STUDIES ON THE INSECTICIDE/NEMATICIDE — OXAMYL AND ITS QUANTITATIVE DETERMINATION BY GAS CHROMATOGRAPHIC METHOD

BY

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A THESIS

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

Brock University

December 1977

Master of Science(1977) Brock University

(Chemistry) St.Catharines,Ontario

Title; STUDIES ON THE INSECTICIDE/NEMATICIDE—— OXAMYL AND ITS QUANTITATIVE DETERMINATION BY GAS CHROMATOGRAPHIC METHOD

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Number of pages; xii, 102

Abstract;

Oxamyl, an insecticide/nematicide with the chemical name; methyl N', N'-dimethyl-N-(methylcarbamoyl)oxy-1-thiooxamimidate, and its major degradation compound; oxime or oximino compound, methyl N',N'-dimethyl-N-hydroxy-1-thiooxamimidate were studied in this work. NMR and mass spectrometry were utilized in the structural studies. An attempt was made to explain the fragmentation patterns of some major peaks in the mass spectra of oxamyl and oxime.

A new gas chromatographic method for the detection and determination of submicrogram levels of intact oxamyl using a electron-capture detector was developed. The principle of this method is to produce a derivative which is highly sensitive to an electron-capture detector. The derivative described is dinitrophenyl methylamine(DNPMA) . Experimental conditions such as pH , reaction temperature , reaction time , the amount of reagent (Dinitrofluoro benzene) etc. were thoroughly investigated and optimized. This method was successfully

applied to the determination of oxamyl residues in tobacco leaves and soil.

Throughout this project , thin layer chromatography was also used in the separation and clean up of oxamyl and oxime samples.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to his supervisor , Dr. M. Chiba , for his guidance,cooperation, and advice during the course of this work and in the preparation of this thesis. The enthusiasm and expertise of Dr. Chiba , not only in science , but also in sports, have a great impact on people around him. It was this enthusiasm that brought us many happy hours playing tennis and badminton together to get away from the occasional boredom of chemistry.

Thanks are also due to Drs. J.M. Miller, J.S. Hartman, and G.R.Finlay for their advice and assistance , and to Dr. A.J.McGinnis, director of the Research Station,Agriculture Canada, Vineland Station, for allowing the use of research equipments and facilities.

The technical assistance of Mr.T. Jones in the NMR and mass spectrometry studies is also acknowledged.

Finally, he wishes to congratulate his wife for successfully keeping him away from the TV set. Without her understanding,advice,assistance and encouragement, this work would have been a more painful task.

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I.A. Pesticide in General

I.A.1. What is a pesticide?

Webster's dictionary defines a pesticide as any chemical that kills insects, weeds, rodents and related pests (1).

The term pesticide branches into many different forms depending on its target of toxic activity. An insecticide is a pesticide that kills insects; a herbicide kills weeds; a fungicide cures or prevents fungus diseases; a nematicide kills nematodes; an acaricide controls mites, and so on.

I.A.2. Historical background

> "The struggle between man and insects began long before the dawn of civilization without cessation to the present time, and will continue, no doubt, as long as the human race endures."..... Wrote Entomologist S.A. Forbes 60 years ago (2).

Despite the mind boggling advances in science and technology over the past decades - the harnessing of nuclear energy, the mastery of space flight, the breaking of genetic code - human kind has made little progress in its age old battle with bugs. For a short period of time after World War II, newly developed chemical pesticides gave scientists hope

that the ultimate weapon against insects had been developed. But, in fact, the bugs were sent temporarily into unprecedented retreat.

The use of pesticides is not new; as far back as 70 A.D., Plinius recommended arsenic as an insecticide and the Chinese regularly used arsenic sulphide for the purpose in the late sixteenth century (3). For the control of aphids, nicotine was first used in 1763 (4). The use of sulfur for controlling the powdery mildew in vines dates back to 1848. Synthetic insecticides were introduced around the same period. One of them - paris green (copper acetoarsenite) was used against the Colorado potato beetle in the United States during the 1860's (2).

The single most significant development in insect control was the discovery of 2,2-bis(p-chlorophenyl)1,1,1-trichloroethane, or DDT (dichloro diphenyl trichloroethane). This compound was synthesized in 1874, but its insecticidal properties were not discovered until 1939 by the Swiss chemist, Paul Mueller. For this work Mueller received the Nobel Prize in 1948 (5). The U.S. Army considered it so effective that it was classified as "Top Secret". Back in Ontario, Canada, DDT was first used jointly by the Ontario Department of Agriculture and the Federal Department of Labour for the control of mosquitoes and house flies in labour camps in 1943. It was also widely used in the control of Dutch Elm disease in elm trees, oriental fruit moth in

peaches, in the control of soil insects such as wireworms and ants, flies in barns and on animals, major pests of potatoes, the Colorado potato beetle, tarnished plant bugs and aphids, as well as insects on peas, beans, corn, cole crops and many others. Due to its wide spectrum of activity and much lower toxicity to mammals, it was widely adopted by the agricultural industry to replace such insecticides as lead arsenate, nicotine sulphate and paris green.

The success of DDT prompted the introduction of BHC and a series of similar chlorine derivatives such as chlordane, heptachlor, toxaphene, endrin, aldrin and dieldrin after World War II. The effects of these pesticides were remarkable. For the first time in history, the world was relatively bug free. One area especially worth mentioning was in the control of diseases carried by pests such as malaria, plague, etc. which cost millions of lives in different parts of the world.

The successful story of DDT and other related pesticides, however, did not last long. In the beginning of the late 40's, scientists began to discover traces of DDT in the tissue of fish, wildlife and humans. The degradation of DDT is relatively slow and DDT is known as one of the persistent insecticides. Most pesticides can undergo faster chemical transformations and degradation. The resulting metabolites are usually less toxic than the parent compounds, but some may be as toxic or more so than

its parent compounds. For DDT, the situation is more complicated. Its major breakdown product, DDE, is nontoxic to insects but is reported to be the cause for eggshell thinning and cracking, increase in embryo mortality, abnormally late breeding and failure to lay eggs in birds (6).

The degradation of DDT was studied by Fleck (7). He reported that in mammals DDT is degraded into 2,2,-bis(p-chlorophenyl) acetic acid (DDA). It is believed that DDA is formed after an initial dehydrochlorination of DDT to DDE as shown in Fig. 1 (8).

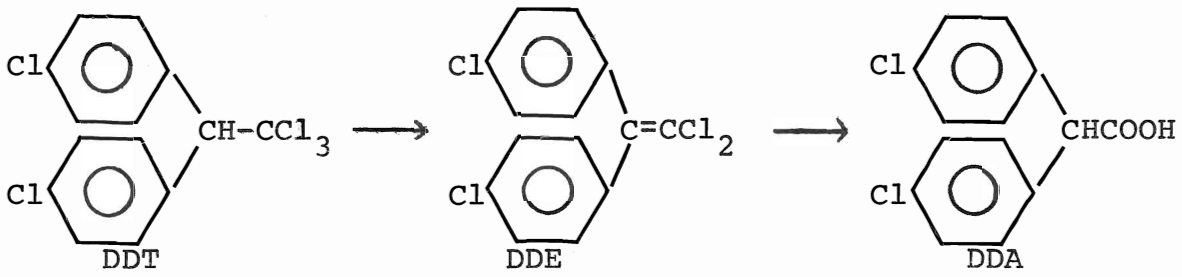


Fig. 1. Dehydrochlorination and hydrolysis of DDT to DDE and DDA in mammals.

Although the breakdown of DDT to DDA is a good feature and takes place extensively, DDT and DDE could be found in most samples of human fat in small quantities. The population of birds such as ospreys, bald eagles and brown pelicans began to decline as a result of insecticide residues in their tissue. It should be noted, however, that there may be other reasons unassociated with DDE or other

compounds for the reduction of bird populations. Bermuda petrel, for example, was reported on the verge of extinction in the late nineteenth century, long before the introduction of DDT or other organochlorine pesticides (6). The anti-insecticide sentiment, nevertheless, was increased further by the publication of Rachel Carson's "Silent Spring". When later studies showed that DDT could cause cancer in laboratory animals, the United States Environmental Protection Agency ordered DDT sales to be restricted in 1972 and banned its use in the U.S. except in cases of sudden serious infestation of epidemics caused by insect vectors (2). The restriction and ban were later also imposed on other insecticides such as *aldrin*, *dieldrin*, heptachlor, chlordane and mirex, all of which were found to be carcinogenic in laboratory animals.

The restriction and ban of these effective pesticides and the development of immunity and resistance to insecticides have since begun to affect the insect populations. In the U.S., the South American fire ant has infested some 150 million acres in nine southern states, injuring and sometimes killing livestock with its fiery sting and driving farm workers from the fields. Experts believe that it will continue to press forward, adapting to cooler temperatures and inexorably moving toward both the north and the west. In forest areas, the spruce budworm, the gypsy moth, the tussock moth and the southern pine beetle are wreaking

devastation on huge areas of woodland, defoliating and killing millions of valuable trees. The spruce budworms also cause a major problem in the forest industries of New Brunswick and other provinces in Canada. Most of these provinces depend heavily on its forest industries. When DDT was abandoned in the spray program for the control of spruce budworms in the late 60's, a new and relatively unknown insecticide, Fenitrothion, was hastily substituted. The opposition to this spraying began when a disease or syndrome called Reye's Syndrome was reported to be related to the spraying in the early 70's. The controversy continues as the spray program to rescue the forest industries continues until the present moment (9).

In Latin America, a bothersome bug; descendant of the high-strung and aggressive "killer bee", is causing widespread concern. Imported from Africa and accidently released in Brazil, where it bred with honeybees of European origin, this fierce hybrid is moving northward at a rate of as much as 200 miles a year. In Argentina, it was reported that the killer bees are on the rampage resulting in the deaths of both man and animals (10).

In other parts of the world, insects are also on the offensive. Malaria, transmitted by mosquitoes and not long ago almost eliminated from many regions, is returning with a vengeance. It strikes 100 million people a year in subsaharan Africa, killing 800,000. River blindness, carried

by a species of blackfly, afflicts a million Africans yearly. The tsetse fly, bearer of sleeping sickness, continue to dominate the continent. Agricultural pests also plague most of the developing nations of Africa.

To counterattack these urgent problems, scientists are left with only two alternatives. They can either resolve to finding new and less persistant pesticides or to utilizing effective biological controls. The latters, regarded as the most promising weapons, can be aimed at specific insect targets without adversely affecting either humans or the environment. Among some of the more novel elements of biological control are; hormones, pheromones, sterilization, development of pest-resistant plants. Experts, however, say that it will be years before biological methods can assume a major role in practical pest control programs. In the meantime, chemical pesticides are essential to maintain the current level of food production (6). In finding new pesticides, attentions have been focused mostly on carbamates, which are more biodegradable and less toxic to nontarget species (11).

I.A.3. Types of pesticides

Pesticides can be subdivided into three main types - inorganic pesticides, naturally occurring pesticides and organic synthetic pesticides.

Inorganic pesticides are copper sulfate, lead

arsenate, paris green (copper acetoarsenite), sodium fluoride, sulfur and others. Naturally occurring pesticides are rotenone, pyrethrum, nicotine, etc. Organochlorines, organophosphorus, and carbamates are the three major groups of organic synthetic pesticides.

I.A.4. Organic synthetic pesticides

Under the three major groups; organochlorine, organophosphorus and carbamates, there are hundreds of synthetic pesticides. Some of the organochlorine pesticides are DDT, BHC, chlordane, heptachlor, aldrin, dieldrin, toxaphene, and endrin. Organophosphorus pesticides include parathion, malathion, dimethoate and others. Carbaryl, carbofuran, oxamyl, methomyl and aldicarb are some of the carbamate pesticides.

Of these three major groups of organic synthetic pesticides, carbamate pesticides are very promising because they are less persistent than organochlorine pesticides and generally less toxic than organophosphorus pesticides. However, studies on carbamate pesticides made to date are not as extensive as those with the other two groups of pesticides.

I.B. Carbamates

The use of carbamate as a poison dated back to the 17th century in west coast Africa. There, a person accused of practising witchcraft was given a solution of calabar

beans to determine his fate (11). Later, the calabar beans were found to contain a carbamate called 'physostigmine', the structure of which is given in Fig. 2.

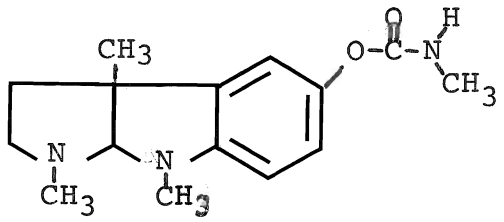
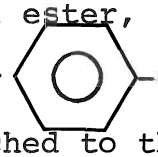
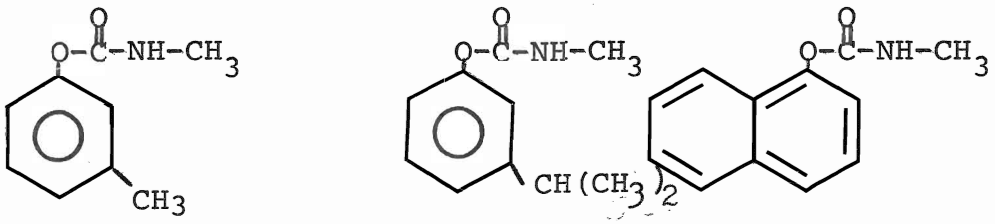


Fig. 2. Physostigmine

The backbone of all carbamate structures is carbamic acid, the monoamide of carbonic acid, $HO-\overset{O}{\overset{\|}{C}}-NH_2$

The acid is unstable and does not exist in free form. It decomposes spontaneously into carbondioxide and ammonia in aqueous media. But salts of this acid, called carbaminates or carbamates, are stable. One such salt is ammonium carbamate, $NH_4^+O-\overset{O}{\overset{\|}{C}}-NH_2$.

Carbamic acid can also be stabilized by forming an alkyl ester, for example, ethyl ester $C_2H_5O-\overset{O}{\overset{\|}{C}}-NH_2$ or aryl ester  . If one of the protons in the latter, attached to the nitrogen is replaced by a methyl group, it is called phenyl N-methyl carbamate, which is mildly toxic. Toxicity increases when substituents are added to the phenyl ring. Most commercial methyl carbamate insecticides are made this way, Fig. 3.



Tsumacide UC 10854 (IPFC) Carbaryl

Fig. 3. Structures of some N-methyl carbamate insecticides.

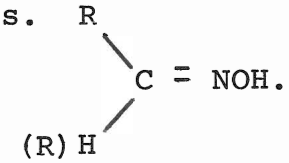
Biologically and chemically, carbamates fall into two general classes. The N-methyl carbamates are used as insecticides and N-aryl carbamates as herbicides (12). Examples of the latters are given in Fig. 4.

I P C or Propham (Isopropyl N-phenyl carbamate)

C I P C or Chloropropham (Chloroisopropyl N-phenyl-carbamate)

Fig. 4. Structures of some carbamate herbicides.

The newest group of methyl carbamate insecticides is derived from aliphatic oximes.



Examples of these oxime carbamates are illustrated in Fig. 5.

Aldicarb Oxamyl

Fig. 5. Structures of two oxime carbamates.

Most pure carbamates are slightly odorous white crystalline solids and they usually have high melting point and low vapour pressure. Most carbamates have excellent shelf stability although they decompose slowly in aqueous solutions. The main degradation pathway of carbamates is hydrolysis which occurs with ease in alkaline media. Resistance to the hydrolysis depends on the chemical structure of the insecticide - namely the substituents on the phenyl ring and the N-substituent. The order of resistance to hydrolysis of N-substituents is— N-phenyl < N-benzyl < N-ethyl < N-methyl (13).

Other factors besides molecular configuration which contribute to carbamate decomposition in alkaline media include temperature, pH and hydroxyl ion concentration (14,15).

Residue analyses for carbamate pesticides are usually more difficult than for other pesticides. For most pesticides, gas chromatography (GLC) is a simple matter once suitable extraction and cleanup methods have been perfected.

Carbamates, however, may not be detected by ordinary GLC even if extraction efficiency and cleanup are excellent, mainly because of decomposition or degradation during gas chromatographic procedures.

Colorimetric methods have been used because of its relative simplicity, availability, reliability and sensitivity. Difficulties most likely encountered with the method are stability of the color complex and interference from co-extractives.

Spectrophotofluorometry has been often used for the residue analysis especially for carbaryl because of strongly fluorescent nature of the naphtholate anion (11).

Even though considerable problems are involved in GLC analysis of carbamate residues, it is obviously the method preferred by most residue chemists. Careful preparation of columns using low polarity methyl and phenyl silicone polymers, modest temperatures, and special column-conditioning procedures have partially solved some of the problems (16,17,18). Some methyl carbamate insecticides can be analysed directly using conventional GLC parameters. For example, Baygon, Zectran and matacil could be chromatographed on an OV-17 column at 180^{o}C (19). Various derivatization methods are also available. Some of these make use of phenol after hydrolysis and some make use of the amine moiety (11). Examples of derivatization methods are, Acylation, Acetylation and Dinitrophenylation (20,21,22,23).

Other methods of analysis are high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), nuclear magnetic resonance (NMR), mass spectrometry (MS), infra-red spectrometry (IR), etc. HPLC proves to be promising because of its mild operating conditions, specificity and possibilities for automation (11).

I.C. Oxamyl and Oximino compound (corresponding oxime)

Oxamyl, Methyl N',N'-dimethyl-N-(methylcarbamoyl)oxy-1-thiooxamimidate, is an insecticide-nematicide discovered by E.I. DuPont de Nemours & Co., Inc. and commercially available as Vydate or previously as DPX 1410. Oxamyl was introduced in 1969 and is registered in the U.S. for early season control of root knot and lesion nematodes in tobacco and for control of various nematodes on certain ornamental and nursery crops (24). It has also been granted approval in France for use on ornamental crops, namely rose bushes and carnations.

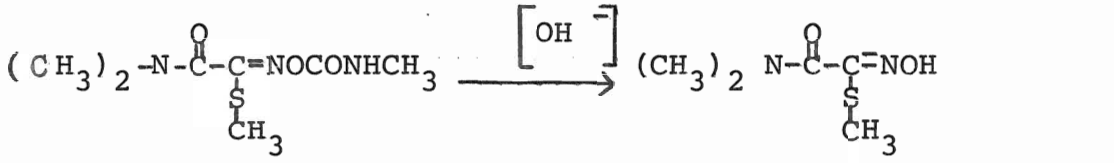
The pesticidal activity of oxamyl is unique. It functions as a systemic miticide/insecticide when applied to the soil. When applied to the foliage of certain plants, it translocates downward to the roots and controls nematodes on some crops (24). This translocation from the leaves to the roots was first reported by Radewald and colleagues (25). Since then it has become increasingly apparent that foliar treatment with Vydate is a promising measure of control for

crops in nematode infested soil. These workers found that Vydate was effective against nematodes in sweet potato, tomato, pumpkin, polebeans and roses. Similar results were subsequently reported by Hart and Maggenti in roses (26); by Abawi and Mai in apple, peach, pear and cherry (27); by Birchfield in cotton (28); by Miller in tobacco (29); by Rich and Bird in tomato and cotton (30); by Taylor and Alphey in cucumber (31); by Jatala and Jensen in peppermint (32); and by Potter and Marks in cabbage (33,34).

These workers were able to demonstrate that oxamyl translocates from the leaves to the roots and that it controls a variety of nematodes in a variety of plants. It was not clear, however, whether oxamyl or a metabolite of it was exuded from the roots after foliar application. Taylor and Alphey postulated that the nematicidal root exudate was a metabolite of oxamyl with a longer half life than that of the parent compound, which is 1-2 weeks (31). They found that when Vydate was applied to the soil, it did not kill all the nematodes present, but that the transmission of virus by these nematodes was stopped. Abawi and Mai did not postulate that there was nematicidal exudate. But they showed that Vydate acted upon nematodes outside the roots and prevented penetration from nematodes (35). Potter and Marks also concluded that the chemical did not kill nematodes in the soil but reduced the numbers of larvae penetrating roots (36).

It is obvious that in order to decide whether the

nematicidal exudate is oxamyl or one of its metabolites, the metabolic pathways of oxamyl have to be investigated. It is now known that the major route of metabolism is through "Hydrolysis". The metabolite formed is the corresponding oxime or oximino compound, methyl N',N'-dimethyl-N-hydroxy-1-thiooxamimidate (37). Fig. 6 indicates the conversion of oxamyl to oxime by hydrolysis.



Oxamyl — Oxime or Oximino compound

Fig. 6. Hydrolysis of oxamyl.

The oxime thus formed is said to be more volatile and yet more stable than oxamyl (38). Holt and Pease developed a method for the determination of oxamyl residues in plants, animal tissues and in soil. The method involved the alkaline hydrolysis of oxamyl to oximino compound and final determination by gas chromotography using a sulfur sensitive flame photometric detector. The method, however, described only the total estimation of oximino compound and not the oxamyl per se. It is worthwhile to develop a residue method that can determine both the oxamyl and oxime individually, particularly by an electron capture detector; by far the most sensitive of all gas chromatographic detectors.

I.D. Scope of Investigation

The initial aim of this investigation was to develop a method that can determine oxamyl and its major metabolite, oximino compound (or oxime) individually in tobacco plants and soil.

A preliminary experiment indicated that oxamyl, like many other carbamates, decomposes on GLC columns. The major decomposed product of oxamyl on GLC columns was found to be an oximino compound which is identical to the major degradation compound found on or in plants after the application of oxamyl. The residue method developed by Holt and Pease gives only a total oximino compound concentration: partly from intact oxamyl which was decomposed during the analysis and the rest from the oximino compound which had existed in the sample and cannot be distinguished from the former. Since the oximino compound was found to be nontoxic to insects and nematodes (39), the determination of oxamyl including a biologically inactive compound will not provide any information regarding the biological significance of residual chemicals. The parent compound, therefore, must be determined separately from the degradation compound. For these reasons, derivatization methods, which are specific to oxamyl, were investigated.

The first derivatization method tried was Trifluoroacetylation. Due to the failure, however, in identifying by mass spectrometer various derivatives produced, this method was abandoned. A second

derivatization method to produce dinitrophenyl compounds was investigated and found to be successful. Sub-microgram levels of oxamyl could be detected in tobacco leaves and soil samples. Both acid and base hydrolyses, and different experimental conditions such as pH, temperature and reaction time were thoroughly investigated to find optimum conditions that will provide good reproducibilities.

Prior to the research work mentioned above, the structures and purities of oxamyl and oximino compound were verified and tested on NMR and Mass Spectrometer. Thin-layer chromatography was also used in the separation of oxamyl and the oximino compound.

II. EXPERIMENTAL

II.A. Study of the Structures of Oxamyl and Corresponding Oxime

The structures of oxamyl and corresponding oxime (or oximino compound), major degradation compound of oxamyl, were studied by mass spectrometry and nuclear magnetic resonance techniques. This study also served to prove the purity of standard chemicals.

II.A.1. Mass spectrometry (MS)

Mass spectrometry has been successfully used for the identification of pesticides. The mass spectra of oxamyl and oxime were thus obtained as follows.

II.A.1.(a). Apparatus and chemicals

The mass spectrometer used was a AEI MS 30 double beam double focusing instrument.

Standard chemicals: Both oxamyl and oxime were obtained from Du Pont Company and were of analytical grades. Oxamyl was 99.9% pure and oxime was 99+% pure.

II.A.1.(b). Study of instrumental conditions

Benzene solutions of oxamyl and oxime, and pure solid oxamyl were used in the mass spectrometric analysis. Direct probe was used to introduce samples.

Ion source: Electron-impact ion source was used since this was the only kind available.

Source temperature: 180°C and 50°C .

Probe temperature: 80-100°C and 50°C.

Ionization potential: Three different ionization potentials, 70 eV, 20 eV and 15 eV were used. Lower values were used along with lower source and probe temperatures when mass spectrometer failed to give a molecular ion peak for oxamyl.

II.A.1.(c) Procedure

For benzene solutions of oxamyl and oxime, a suitable volume of the solution was taken in a syringe and transferred into a small vial. The solvent was allowed to evaporate. Then, the vial containing residue was introduced into the mass spectrometer via direct probe. For solid samples, a few grains of the sample was taken in the vial and inserted via direct probe.

II.A.2. Nuclear magnetic resonance (NMR)

Proton magnetic resonance, ^{1}H NMR of pure oxamyl and oximino compound was investigated.

II.A.2.(a) Apparatus and chemicals

Apparatus: Bruker WP-60 NMR spectrometer was used for the NMR study.

Chemicals: Deuterated water and acetone supplied by

Merck Sharp and Dome Canada Ltd., Kirkland, Quebec were used.

II.B. Separation of Oxamyl and Oxime

II.B.1. Thin layer chromotography

Thin layer chromatography (TLC) has been widely used as a microanalytical tool for pesticides. Many papers have been published on this subject since its discovery (40). Beroza utilized TLC for the separation and subsequent identification of the methylenedioxyphenyl synergists that were commercially used to enhance the biological activity of pyrethroids (41). Fischer and Klingelholler developed both qualitative and quantitative TLC procedures for the determination of several phosphorous pesticides in body fluids (42). Morley and Chiba used TLC for the determination of chlorinated pesticide residues in some plant extracts without prior cleanup. The importance of this study is in the elimination of possible loss of pesticides during the cleanup process (43).

II.B.1.(a). Apparatus and reagents

Developing jar: 500 ml capacity with screw type lid.

TLC plate: Fluorescent indicator treated silica gel sheet supplied by Eastman Kodak Co., Rochester, N.Y., 14650 silica gel (no. 6060).

UV light source: Black Ray UVL-22 long wavelength supplied by Ultra-violet Products Inc., Sangabriel, California.

Micropipettes: one and 2 µl capacity, microcaps, disposable type supplied by Kensington Scientific Corporation, California.

Solvents: glass distilled acetone, ethyl acetate and benzene were supplied by Caledon Laboratories Ltd., Canada and Burdick and Jackson Laboratories, Inc.

II.B.1.(b). Choice of developing agents

Four different developing solvents were tried. They were acetone, ethyl acetate, benzene and a mixture of ethyl acetate and benzene (1:1).

II.B.1.(c) Procedure

Ten milliliters of one of the above solvents was added to the developing jar. It was then tightly closed with a lid and shaken to saturate it with vapour from the solvent.

A strip of TLC plate (5 x 10 cm) previously washed with ethyl acetate by the same TLC was used. Solutions of oxamyl and corresponding oximino compound in benzene (1 mg/ml) were spotted on the plate by the use of micropipettes. The plate was developed in the jar, dried and spots were then observed under UV lamp.

II.B.2. Gas liquid chromatography (GLC or GC)

II.B.2.(a) Apparatus and reagents

Gas chromatograph: Varian Aerograph model 1200 equipped

with an electron capture (tritium foil) detector and a Varian model 20 recorder.

Syringe: 10 µl capacity 701N Hamilton syringe.

Column: 90 cm x 3 mm I.D. glass column packed with 3% XE-60 on Gas Chrom Q 80/100 mesh.

II.B.2(b) Preliminary studies

Different kinds of stationary phases, namely 3% SE-30, 3% OV-3, 3% XE-60 and a mixed phase of 11% QF-1 and OV-17, all coated on Gas Chrom Q 80/100 mesh, were investigated.

A wide range of column temperatures from 150°C to 210°C were also studied.

II.B.2.(c) Procedure

Three to five microliters of the 10 ppm oxamyl and corresponding oxime solutions were injected separately into GC columns and under different column temperatures such as 150°C, 165°C, 180°C, 195°C and 210°C. Injector port temperature was maintained at about ten degrees higher than the column temperature used. The detector temperature used was 220°C. Flow rate of nitrogen carrier gas was maintained at about 35 - 40 ml per minute. Retention times were measured by time lapse or by distance between the solvent front and the peaks for the chemicals.

II.C. Development of a Method for the Analysis of Oxamyl

II.C.1. Trifluroacetylation

II.C.1.(a) Apparatus and chemicals

Apparatus: Apparatus used was the same as that mentioned in section II.B.2(a).

Chemicals: Trifluoro acetic anhydride of reagent grade was supplied by Eastman Kodak (No. 7386) and was 98+% pure. Pyridine, also of analytical grade, was from British Drug House.

Solvents used were glass distilled pesticide grade from Caledon Laboratories Ltd. and Burdick and Jackson Laboratories, Inc.

II.C.1.(b). Procedure

The trifluoroacetylation method developed by Lau and Marxmiller (44), and Ueji and Kanazawa (45) was modified as follows.

To a 10 ml graduated test tube provided with a screw cap, 0.2 ml of a 10 ppm solution of oxamyl or corresponding oxime in ethyl acetate was added. Then, 0.1 ml of pyridine and 0.1 ml of trifluoroaceticanhydride were added. The tube was stoppered, mixed by shaking and was kept in a water bath at 25^{o}C for 15 minutes. A mixture of n-hexane and ether (4.5 ml and 0.3 ml respectively) was then added. The content was then transferred to a 100 ml separatory funnel and washed with 5 ml of distilled water for three times and the washings were discarded. The volume of organic solvent left was made up to

5 ml with hexane. About 0.2 gram of anhydrous sodium sulphate was then added and the sample was ready for GC injection.

The samples thus prepared were also run on GC-MS unit.

II.C.2. Dinitrophenylation

II.C.2.(a). Apparatus and chemicals

Basic apparatus and solvents used were the same as those used in section II.C.1.(a).

Votex-Genie vibrator: supplied by Fisher Scientific.

Rotary vacuum evaporator: Rotavapor (R) supplied by Buchi, Switzerland.

Temperature controller: Fail safe tempunit (R) by Techne Chembridge Ltd., England.

Dinitrofluorobenzene (DNFB) was supplied by Eastman Kodak.

Glycine and other chemicals used such as sulphuric acid (ACS grade), sodium carbonate, sodium hydroxide and sodium borate were of reagent grades.

II.C.2.(b). Basic hydrolysis and dinitrophenylation

This method was first introduced by Day et al in the determination of microquantities of the C1 to C4 primary and secondary amines (46). It was later used by Mendoza and Shields (47), and Sumida et al (48) for the determination of carbamate residues in plant materials. The present study utilized the

optimum conditions for the determination of oxamyl. These conditions have been established as a result of extensive study to find significance of different experimental parameters, such as, pH, temperature, time factor, different kinds of base and amounts of dinitrofluorobenzene.

Five milliliters of pH 12 buffer solution (consisting of 50 ml of 0.05M Na_2HPO_4 and 26.9 ml of 0.1M sodium hydroxide) was added to a 15 ml screw capped graduated centrifuge tube containing oxamyl aqueous sample or methylamine hydrochloride aqueous solution. The solution was shaken on a vortex vibrator for a minute and heated for ten minutes in a 80°C water bath which temperature was controlled by a fail safe tempunit. One milliliter of 1% DNFB in dioxane was added to the solution and allowed to react in the 80°C water bath for ten minutes. Then, one milliliter saturated glycine solution was added and the solution was heated again for ten minutes. The tube was then cooled in ice bath. The reaction product, dinitrophenyl methylamine (DNPMA) was extracted with 4 ml of benzene. The extraction was repeated with 2 ml of benzene and the extracts were combined. The combined benzene extract was washed with 7 ml of 0.1 N sodium carbonate solution. The sodium carbonate layer was washed with 4 ml of fresh benzene. The combined benzene was dried by adding about 0.2 gram of anhydrous sodium sulphate and was made up to 10 ml or 25 ml; the solution was ready for GC injection. Dilutions had to be made for samples

of high concentration.

II.C.2.(c). Preparation of standard DNPMA and dinitrophenyl dimethylamine (DNPDMA).

This method of preparation for dinitrophenylamines was first used by Day et al (46). About one gram of amine was dissolved in 50 ml of borate buffer solution (2.5% aqueous solution of sodium borate); to this solution DNFB (2 ml in 25 ml of 1,4-dioxane) was added and the mixture was heated on a steam bath for one hour. After adding 50 ml of 2 N sodium hydroxide solution the mixture was filtered, the crystal was washed with 0.1 N sodium carbonate and recrystallized from ethanol-water or petroleum ether-cyclohexane. The lower melting DNPDMA did not solidify in the reaction mixture. It was extracted with hexane, washed with 0.1 N sodium carbonate, dried with anhydrous sodium sulfate and evaporated to dryness on a rotary vacuum evaporator. The residue was recrystallized from petroleum ether-cyclohexane. These were used for references.

II.C.2(d). Acid hydrolysis and dinitrophenylation

Acid hydrolysis of oxamyl was also investigated. The amount and the kind of acid used were those recommended by Tilden and Van Middelem in their derivatization method by 4-bromobenzoyl chloride (49). Reaction time, temperature, different ways of adding DNFB were further investigated.

One milliliter of aqueous sample solution was taken in a 15 ml screw capped centrifuge tube and 0.5 ml of concentrated sulphuric acid (ACS grade) was added. The mixture was heated in a 82°C water bath for 10 minutes. After cooling, one milliliter of 1% DNFB in dioxane (or in benzene) was added to the mixture. Sodium hydroxide solution (5N) was added until strongly alkaline. The sample was then transferred to a separatory funnel and extracted with 4 ml followed by 2 ml of benzene.

II.D. Dinitrophenylation of Oxamyl on Tobacco Leaves

II.D.1. Fortification of oxamyl on tobacco leaves and its extraction

II.D.1.(a) Apparatus and chemicals

Omni mixer: supplied by Ivan Sorvall Inc., U.S.A.

Micropipettes: different capacity from 2 to 20 µl, supplied by Kensington Scientific Corporation, California.

All other apparatus, chemicals and solvents were the same as described previously in section II.C.1.(a).

II.D.1.(b) Procedure

A sample of tobacco leaves (10g) was taken in a 400 ml Omni-Mixer container and a known amount of oxamyl aqueous solution was added with a micro pipette. Then, 50 ml of ethyl acetate was added and the contents were blended at high speed

for five minutes. The mixture was filtered through a bed of anhydrous sodium sulphate into a 250 ml round bottom flask. The extraction was repeated two more times, each time using 50 ml of ethyl acetate and the extracts were combined. Twenty milliliters of water was then added to the combined extract. The ethyl acetate was later evaporated at 60°C. The residual water phase was transferred to a 125 ml separatory funnel and repeatedly washed with hexane until the hexane layer almost became colourless. The washings were discarded. The aqueous layer was then ready for derivatization as shown in section II.C.2.

Two varieties of tobacco leaves were used in the experiment, namely Harrow vel, grown in the greenhouse of Vineland Research Station, Agriculture Canada and Delhi-34, obtained from Delhi Research Station, Agriculture Canada.

II.D.1.(c) Clean up by thin layer chromatography

Because of the interfering peaks from tobacco leaves which showed up in chromatogram, the derivatized samples were first cleaned up by thin layer chromatography. The best developing solvent found was a mixture of ethyl acetate and hexane (1:1). The apparatus, chemicals used and the procedure were the same as those discussed in section II.B.1.

After developing, the spots corresponding to the standard DNPMA spot were cut off, extracted with benzene and

then injected into GC.

II.D.1.(d) Gas liquid chromatography (GLC)

The following GLC conditions were utilized in the analysis.

Gas chromatograph: Varian aerograph model 1200, equipped with a tritium electron capture detector.

Column: 1.82 m x 2 mm I.D. glass column.

Stationary phase and support: 3% XE-60 on Gas Chrom Q, 100/120 mesh.

Injector temperature: 220°C.

Column temperature: 210°C.

Detector temperature: 220°C.

Carrier gas: high purity dry nitrogen.

Flow rate: 37 milliliters per minute.

Recorder: Varian model 20.

II.D.2. Determination of oxamyl in field tobacco samples

II.D.2.(a). Apparatus and chemicals

Apparatus and chemicals were the same as those described in section II.D.1.(a).

Tobacco leaves sprayed with oxamyl were obtained from the Agriculture Canada experimental farm located in Jordan Station. Blank tobacco leaves were obtained from greenhouses in Vineland Research Station, Agriculture Canada.

II.D.2.(b). Procedure

A ten gram tobacco leaf sample was taken in a Omni-Mixer and 50 ml of ethyl acetate was added. The rest of the procedure was similar to that of section II.D.1.(b).

II.D.3. Determination of oxamyl in field soil samples.

II.D.3.(a) Apparatus and chemicals

All the apparatus and chemicals used were the same as those described previously except the following.

Glass container: 450 ml capacity, with screw capped lid.

Shaker: Fisher-Kendall mixer.

Soil: Sandy loam soil on which oxamyl-sprayed tobacco plants were grown, collected at the Agriculture Canada experimental farm in Jordan Station.

Blank sandy loam soil was obtained from greenhouses in Vineland Research Station, Agriculture Canada.

II.D.3.(b). Procedure

Moisture content of the soil sample was analysed before the determination of oxamyl in soil. Water was added to the sample if necessary to bring the moisture content up to 40% to improve the extraction efficiency.

A sample of 50 gram soil was weighed in a jar and its moisture content adjusted. One hundred ml of ethyl acetate

was added and the mixture was tumbled for 30 minutes on a Fisher-Kendall mixer. This extraction was repeated with 50 ml of ethyl acetate. The soil and container were washed with 40 ml of ethyl acetate. The ethyl acetate extract including the washing was transferred through a filter paper to a round bottom flask of the rotary vacuum evaporator and 20 ml of water was added. The ethyl acetate was then evaporated at 60°C. The volume of water layer was adjusted to 20 ml.

Ten milliliters of the aqueous layer was taken and derivatized with DNFB according to the procedure described in section II.C.2.(b). The derivatized product in benzene was ready for GC injection.

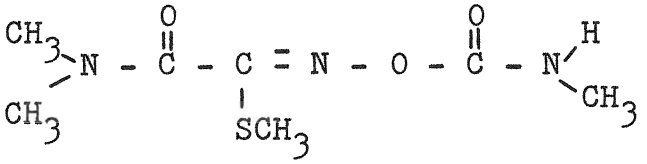
III. RESULTS AND DISCUSSION

III.A. Study of the Structures of Oxamyl and Corresponding Oxime

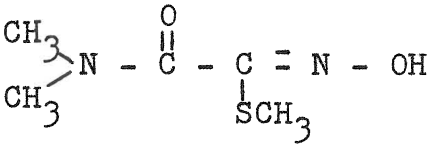
At the beginning of this project, oxamyl and its corresponding oxime were studied by physical methods such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) . Structural studies and subsequent identification of pesticides by MS and NMR have been proved to be indispensable for a pesticide chemist. These studies could also provide information regarding the purity of the chemicals.

III.A.1. Mass Spectrometry (MS)

As mentioned previously, the structures of oxamyl and its corresponding oxime are given as follows.



oxamyl



oxime

Mass spectra of benzene solutions of oxamyl and oxime were first analysed by evaporating the solutions in solid probe vials. The concentrations were 1000 ppm for oxamyl and 100 ppm for oxime. Instrumental conditions for these analyses

were as follows.

source temperature 180° C

probe temperature 80°C - 100°C

sensitivity 8.5

energy 70 eV

sample size 5.0 µl

Under these conditions both oxamyl and oxime gave very similar spectra (Fig. 7 and 8). A peak at m/e 162 which corresponded to the molecular weight of oxime was observed on both spectra. However, a molecular ion peak at m/e 219 for oxamyl was not observed. This indicated that oxamyl was decomposed to oxime either in the benzene solution or during the mass spectrometry procedure. Since a subsequent TLC analysis proved that oxamyl was still intact in benzene at the time of sample injection to the MS system, it was concluded that the degradation of oxamyl took place during the MS procedure.

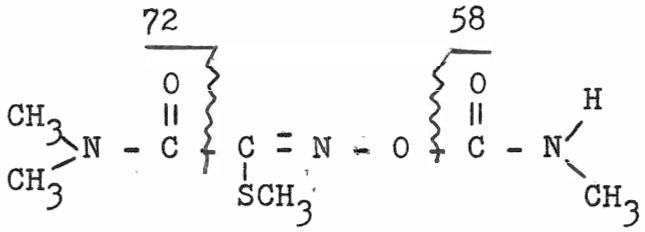
Milder conditions, therefore, were next investigated in an attempt to obtain a parent peak of m/e 219. Both the source and probe temperatures were lowered to 50°C and the energy was decreased to 15 eV. Even at these conditions , however, no parent peak at m/e 219 was observed.

Then pure oxamyl crystals (supplied by Du Pont) instead of the benzene solutions were analysed, but no parent peak of m/e 219 was obtained even by employing a low temperature of 50° C and energy of 20 eV. The absence of its molecular ion peak

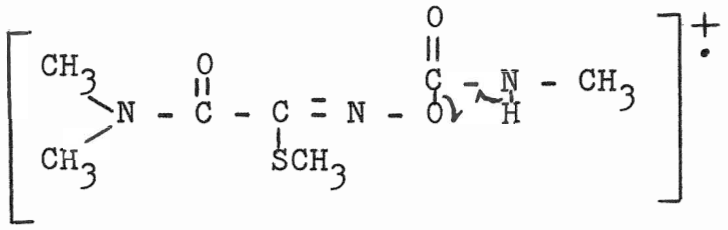
suggested that oxamyl is heat labile . It was found later that Reiser and Harvey Jr. of Du Pont had succeeded in obtaining a very weak molecular ion peak at m/e 219 with an all glass system (37).

The mass spectra of oxamyl and oxime obtained by AEI MS 30 mass spectrometer are shown in Fig. 7 and 8.

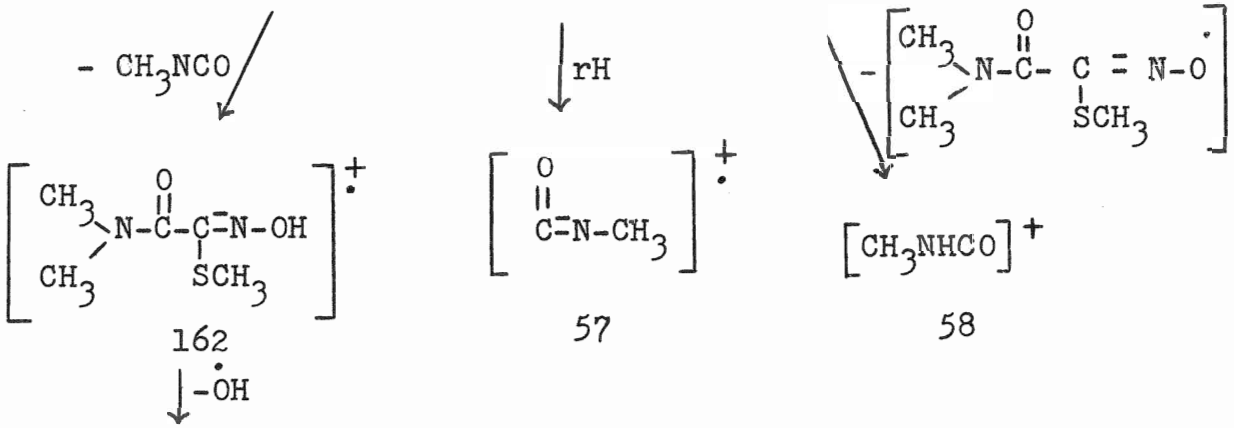
The major fragmentations pattern of oxamyl (for oxime, fragmentations begin from m/e 162) is assumed to be as follows.

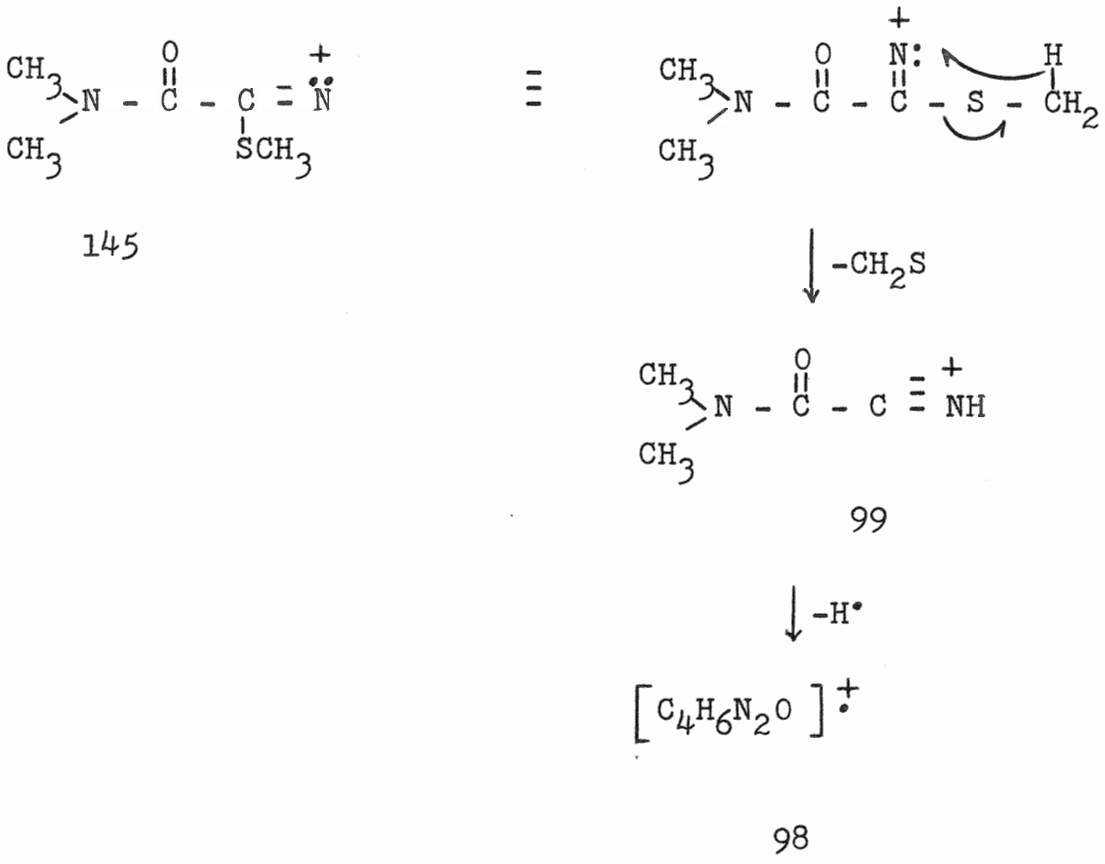


III



m/e 219





145

99

98

Since the mass spectrometer failed to give a molecular ion peak for oxamyl , the identification of oxamyl by this means was judged to be difficult . It would be of interest to determine if chemical ionization methods could yield a molecular ion peak for oxamyl.

III.A.2 *Nuclear Magnetic Resonance*

The NMR investigation of oxamyl and oxime was successful. Fig.9 shows the NMR spectrum of oxamyl. Deuterated water was used as a solvent for oxamyl and Trimethyl Silylpropionate (TSP) was used as an internal standard. The doublet at 3.05 ppm is

Fig. 7. Mass spectrum of oxamyl

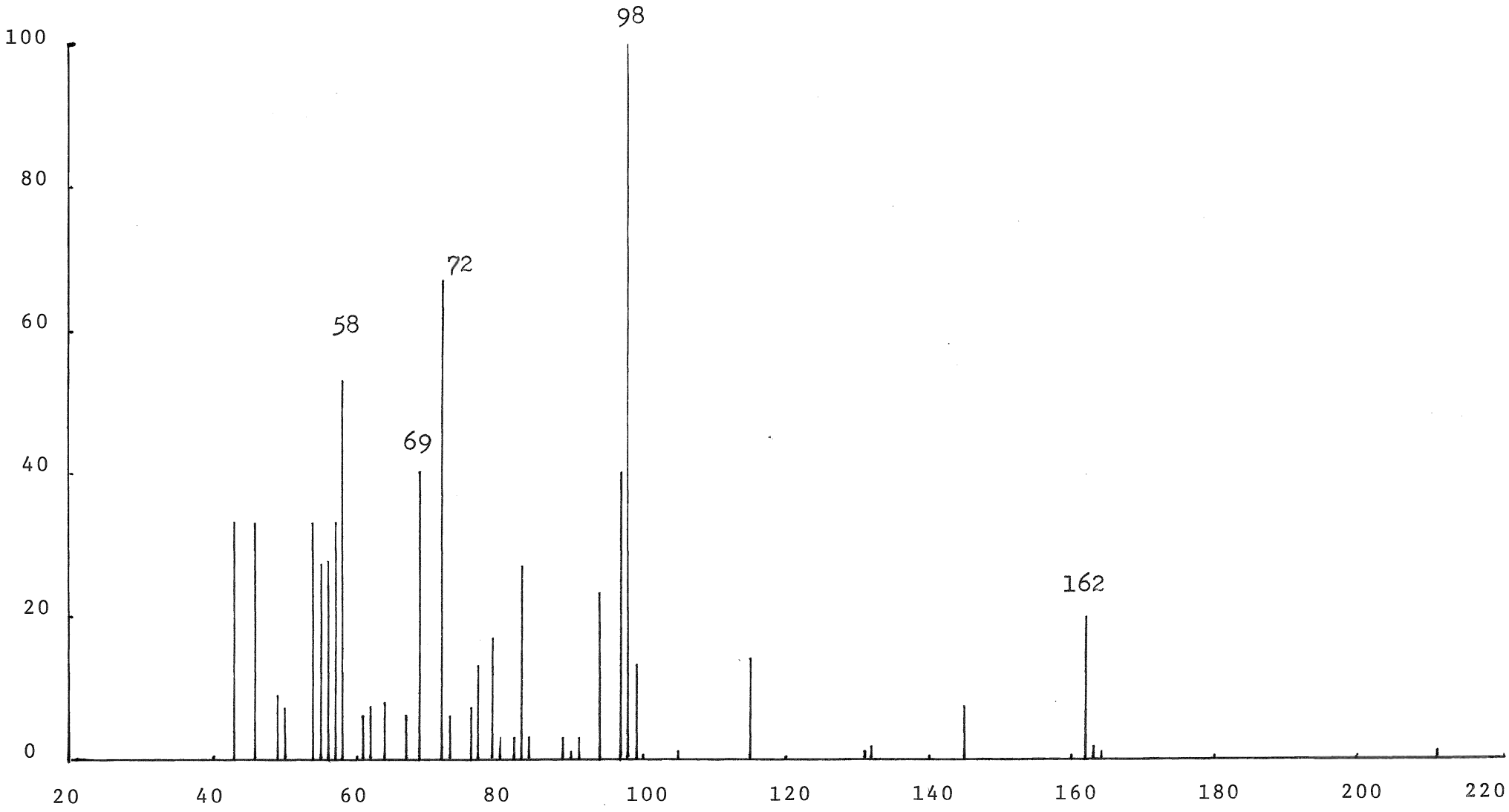

98
100
80
72
60
58
69
40
20
162
0
20
40
60
80
100
120
140
160
180
200
220

Fig. 8. Mass spectrum of oxime

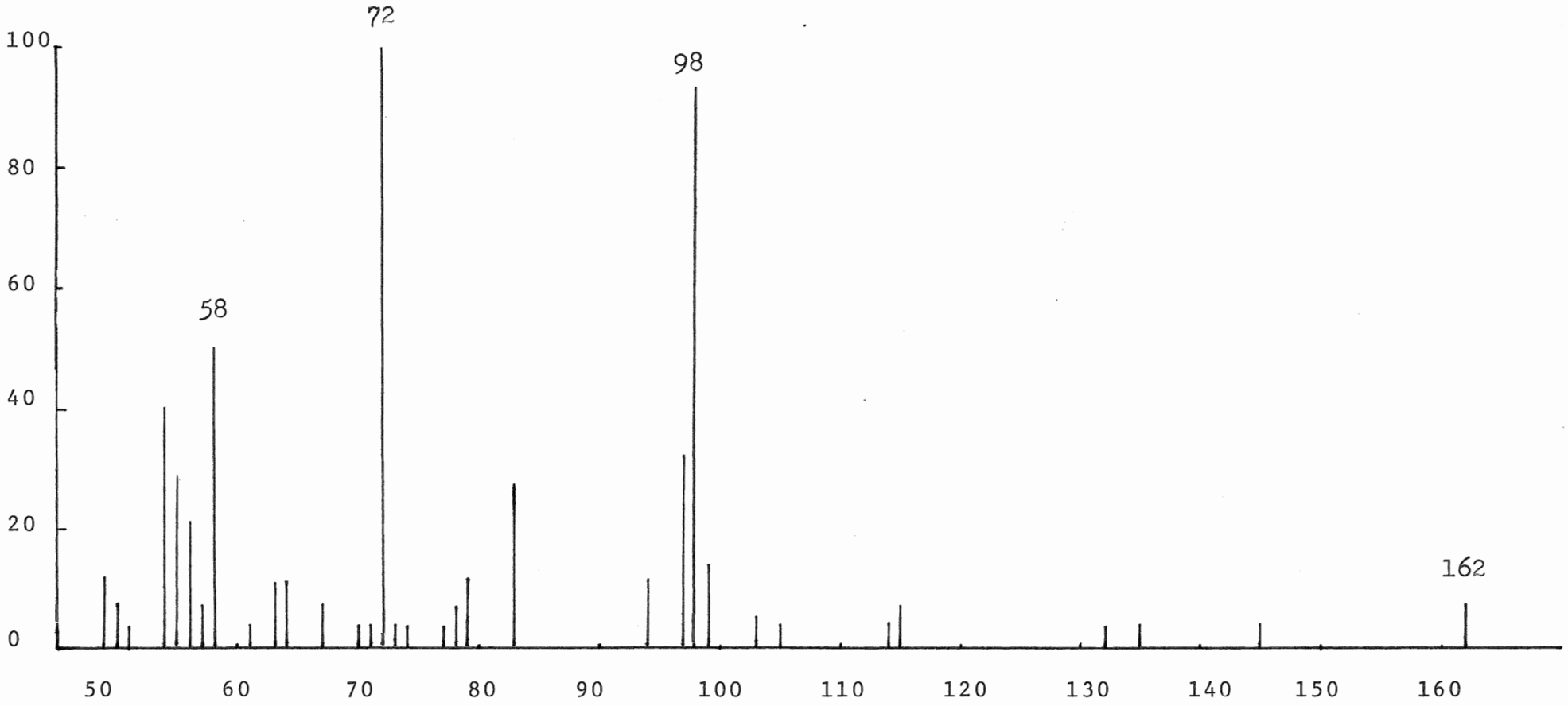

72
98
58
162
100
80
60
40
20
0
50
60
70
80
90
100
110
120
130
140
150
160

Fig. 9. NMR spectrum of oxamyl (^{1}H)

BRUKER

60 MHz ^{1}H Spectrum

Spec. No. 1
No. of scans 16
SW 750 Hz/cm
Points 8 k / 4 k
PW 1.1 μ sec. Rep. 5.5 sec.

SAMPLE OXAMYL

Solv. + Lock (D)
- ☑ D_2O pH .....
- ☐ $CDCl_3$
- ☐ d_6-DMSO
- ☐ C_6D_6
- ☐ CD_3COCD_3
- ☐ .....
- ☐ .....

TMS
- ☑ int. (TSP)
- ☐ ext. cap.
- ☐ ext. calc.

Decoupling
- ☐ normal
- ☐ gated

Freq. ..... Hz

Temp.
- ☑ amb.
- ☐ ..... °K

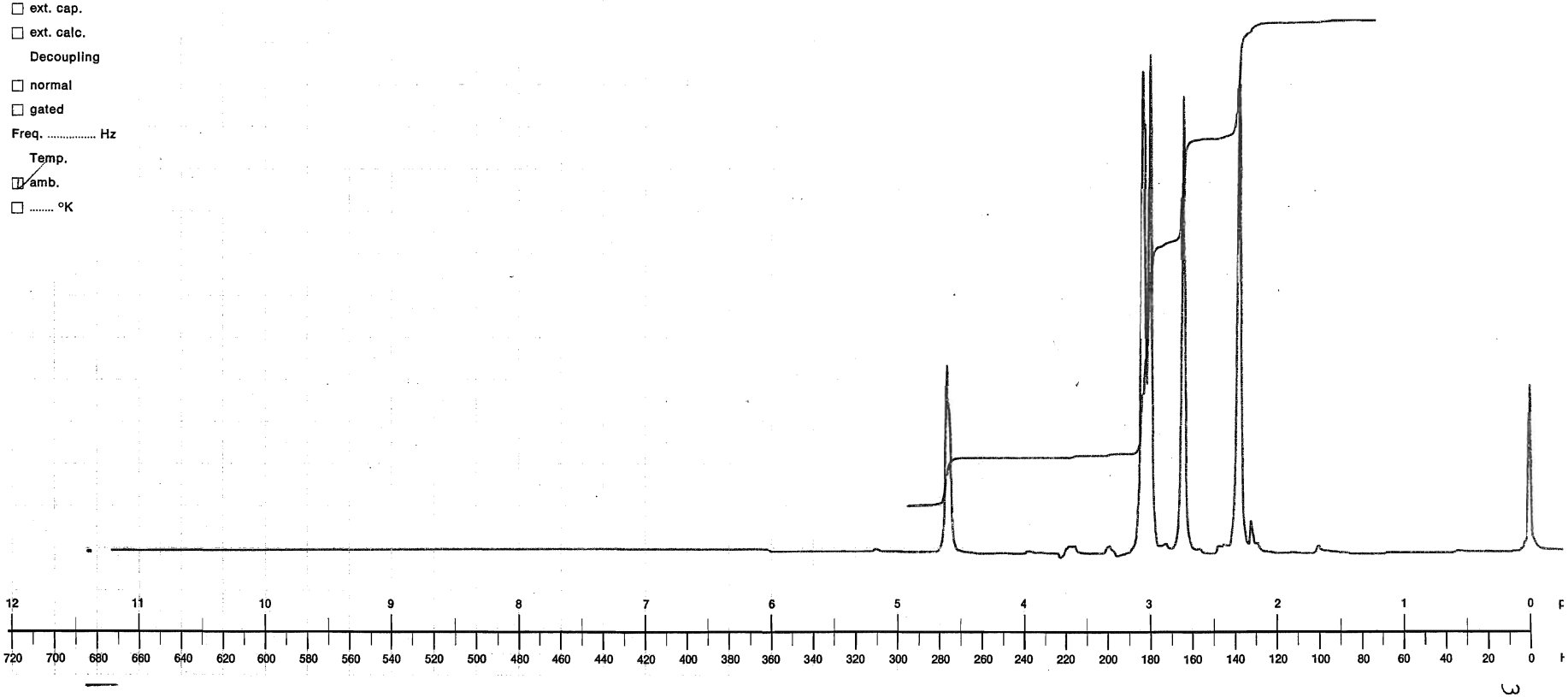


Fig. 10. NMR spectrum of oxime (^{1}H)

BRUKER

60 MHz ^{1}H Spectrum

SAMPLE OXIME

Solv. + Lock (D)

- ☐ D_2O pH .....
- ☐ $CDCl_3$
- ☐ d_6-DMSO
- ☐ C_6D_6
- ☑ CD_3COCD_3
- ☐ .....
- ☐ .....

TMS

- ☑ int.
- ☐ ext. cap.
- ☐ ext. calc.

Decoupling

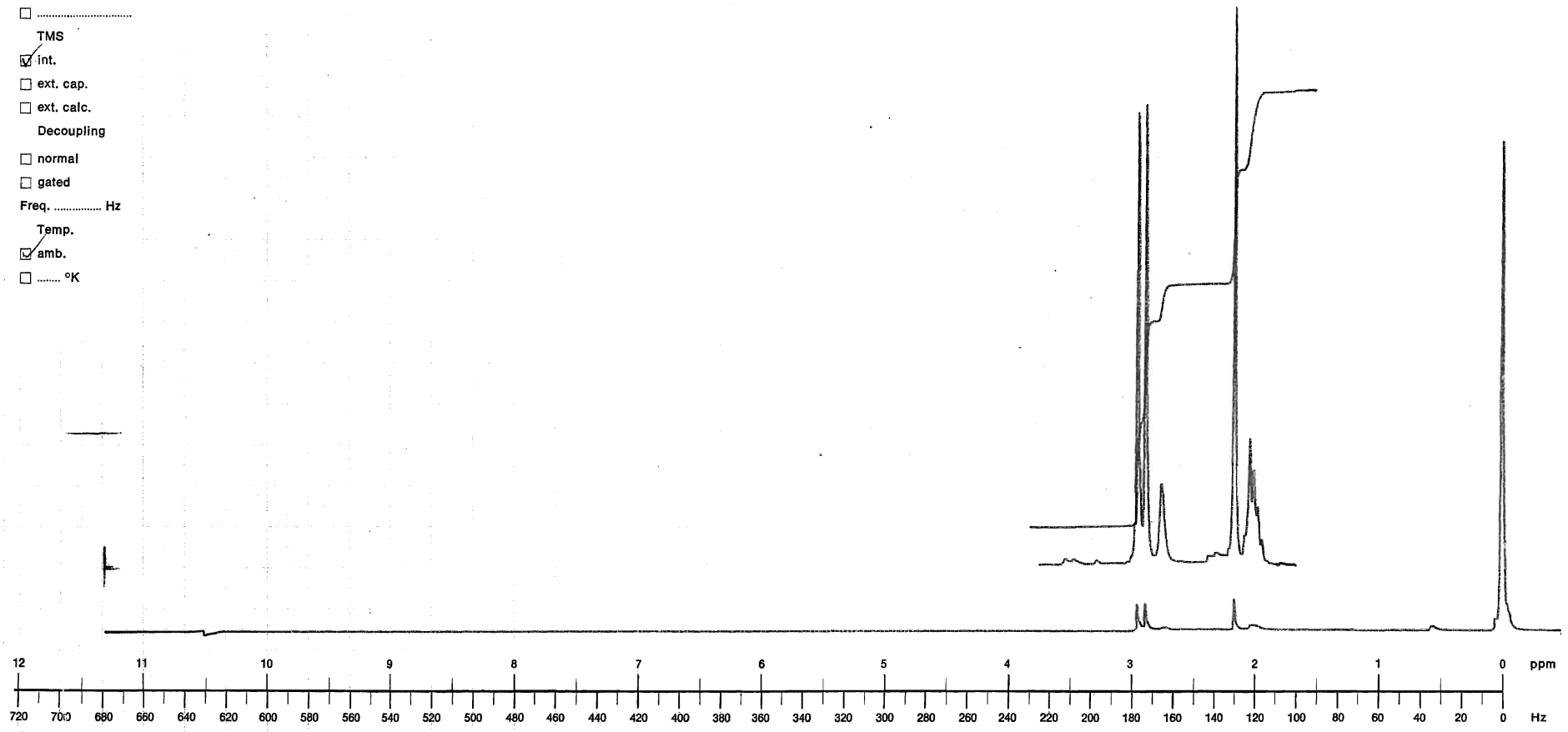
- ☐ normal
- ☐ gated

Freq. ..... Hz

Temp.

- ☑ amb.
- ☐ ..... °K

Spec. No. 2
No. of scans 16
SW 750 Hz/cm
Points 8 k / 4 k
PW 4.1 μ sec. Rep. 5.5 sec.



due to the $(CH_3)_2$-N proton. The singlets at 2.75 ppm and 2.3 ppm represent N-CH_3 protons and S-CH_3 protons respectively. The singlet at 4.6ppm is due to HDO/H_2O.

The NMR spectrum of oxime is shown in Fig. 10. The ($CH_3)_2$-N protons are represented by a doublet at 2.95 ppm. The singlets at 2.15 ppm and 2.75 ppm represent the -SCH_3 and -OH protons respectively. Deuterated acetone was used as solvent and TMS (tetramethyl silane) was used as internal standard.

NMR therefore can be utilized for the differentiation of oxamyl and oxime. At the same time, oxamyl and oxime samples obtained from Du Pont were proved to be pure.

III.B. Separation of Oxamyl and Oxime

III.B.1. Thin Layer Chromatography

The very first experiment performed involving TLC was to investigate whether oxamyl and oxime were detectable at a reasonable sensitivity. Acetone was used as a developing solvent in this experiment. Two microliters, one microliter and 0.5 microliter (approximate) of 1000 ppm oxamyl in benzene stock solution equivalent to 2 µg, 1 µg and 0.5 µg of oxamyl were spotted on a prewashed (with ethylacetate) TLC plate. After developing , the plate was viewed under UV light. The same experiment was repeated for oxime. Both the compounds gave dense spots with slightly different R_f values and the minimum detectable amount was 1 µg for each compound.

To choose the best solvent for the separation of oxamyl and oxime , four different solvent systems were investigated.

They were acetone, ethyl acetate, benzene and ethyl acetate: benzene (1:1). The results of these experiments are recorded in Table 1.

As a result , the best solvent for the separation of oxamyl and oxime by TLC was judged to be ethyl acetate . Both oxamyl and oxime gave intense, defined spots without any tailing . The distance between two spots is sufficiently wide for good separation .A sample of two typical TLC plates is shown in Fig.11.

In the course of this TLC study , oxamyl was found to be unstable in benzene after a certain period. A 1000 ppm oxamyl benzene solution was prepared and the solution was kept at room temperature. About 100 days later, 2 µl sample of the solution was spotted on a TLC plate . After developing , two spots were visible under UV lamp; these R_f values were identical to those of oxamyl and oxime . This clearly indicated that oxamyl was not stable in benzene solution (over long periods, eg. 100 days) and to a certain extent degrade to its corresponding oxime. It was found that a similar degradation also took place in water. John Harvey , Jr. of Du Pont reported a similar degradation in plants and soil (39, 50).

III.B.2. Gas-Liquid Chromatography

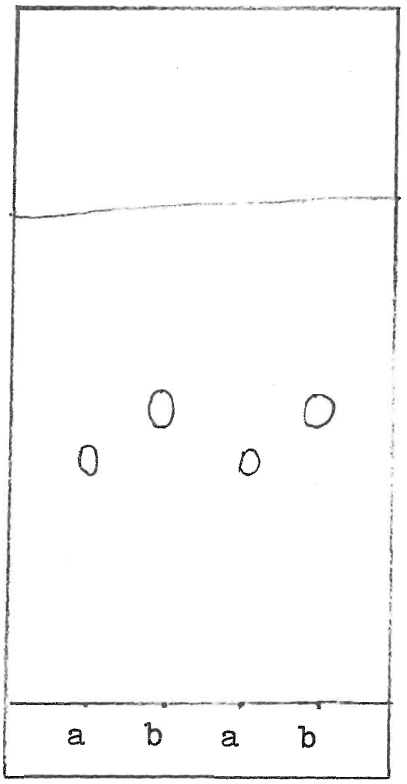
Gas-liquid chromatography is an efficient and rapid method for the separation , identification and quantitation of components from small batches of mixture of volatile compounds.

Table 1

R_f values of oxamyl and corresponding oxime in different solvents

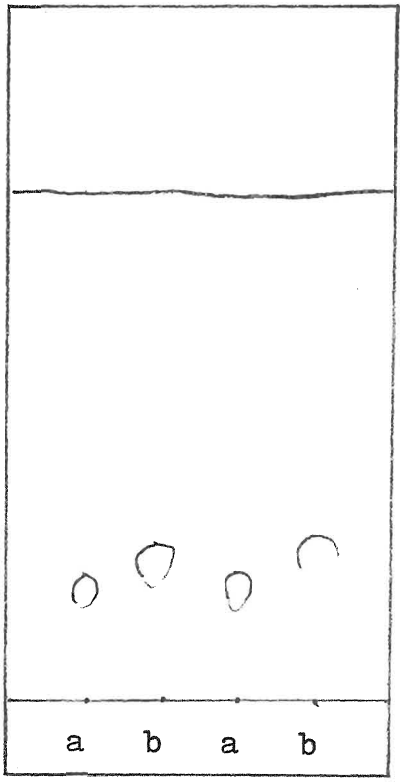
Solvent	R_f values of oxamyl	R_f values of oxime
Acetone	0.83	0.83
	0.83	0.83
	0.81	0.85
Benzene	0.00	0.00
Ethyl acetate/ Benzene(1:1)	0.23	0.31
	0.23	0.32
Ethyl acetate	0.52	0.62
	0.51	0.61

Fig.11. TLC plates of oxamyl and oxime in two developing solvent systems



Developing solvent =

ethyl acetate



Developing solvent =

ethyl acetate; benzene (1:1)

a = oxamyl

b = oxime

Introduced by James and Martin in 1952, the method was rapidly put to use in the petroleum industry and other fields of chemistry and biology (51). The list of problems that can be solved by gas chromatography is enormous; some have been rather exotic, like the investigation of the sex attractants secreted by insects, and of the aroma of coffee and of many other flavors. In short , it is one of the most widely used analytical techniques in all branches of chemistry.

In pesticide residue analysis, gas chromatography has proven to be one of the most valuable tools. Depending on the type of detector, pesticide residues as low as nanogram (10^{-9} gm) or even picogram (10^{-12} gm) level could be detected by gas chromatography.

In an attempt to separate and identify oxamyl and its corresponding oxime with a gas chromatograph, benzene solutions of the individual chemicals were injected. Of the four different liquid phases investigated, namely, 3% SE-30, 3% OV-3, 3% XE-60 and a mixed phase of 11% QF-1 and OV-17, the one found to be most suitable was 3% XE-60. It gave a reasonable retention time and peak shape to the oxime. The operating parameters were---column temperature 150°C , injector temperature 180°C , detector temperature 220°C , and nitrogen carrier gas flow was 40 ml/min. Under these conditions , however, oxamyl like most carbamate insecticides, was found to be decomposed to other compounds. Its major product was corresponding oximino compound. Further verifications

were made by altering the GC conditions such as column temperature and flow rate . Without exception , oxamyl gave a peak which retention time was identical to its corresponding oxime (see Fig. 12) . This finding prompted an investigation for a new method that can detect oxamyl and oxime individually.

III.C. Development of a method for the analysis of oxamyl

III.C.1. Trifluoroacetylation

Trifluoroacetylation was one of the indirect or derivatization methods investigated when direct method of detecting oxamyl was found unsuccessful.

The indirect method was first reported by Lau and Marxmiller in 1970 to detect nanogram level of Landrin in corn (44). This method utilized trifluoroacetic anhydride to convert Landrin to an electron-capturing derivative. The reaction mixture was required to be left overnight for completion. Ueji and Kanazawa modified and shortened the reaction time considerably by adding pyridine (45) and warming the mixture. The reaction of trifluoroacetic anhydride with Landrin insecticide is as follow ;

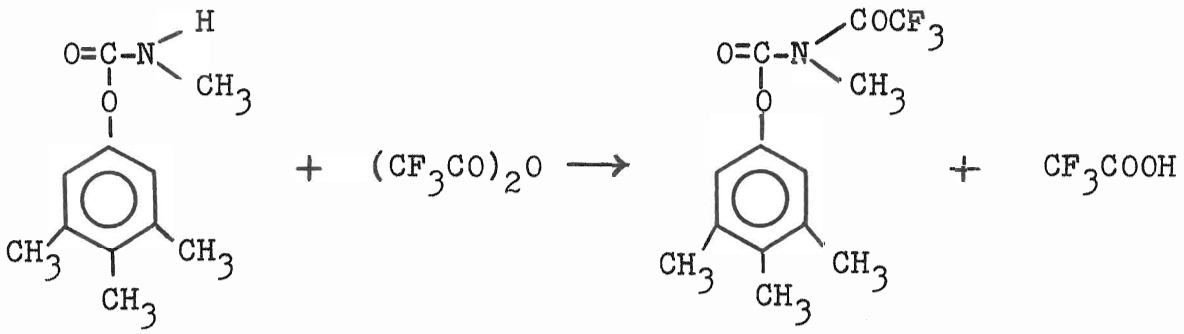
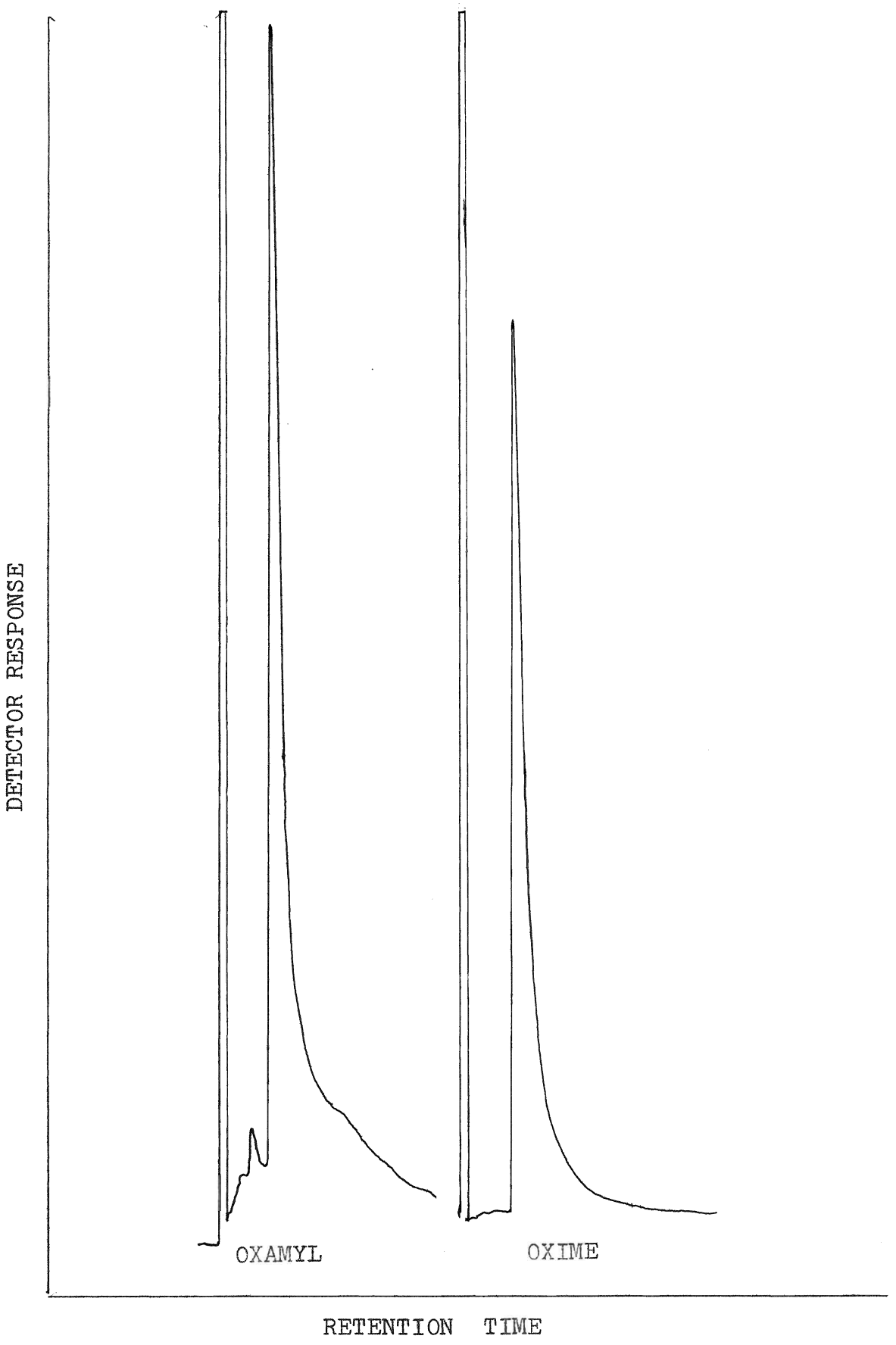
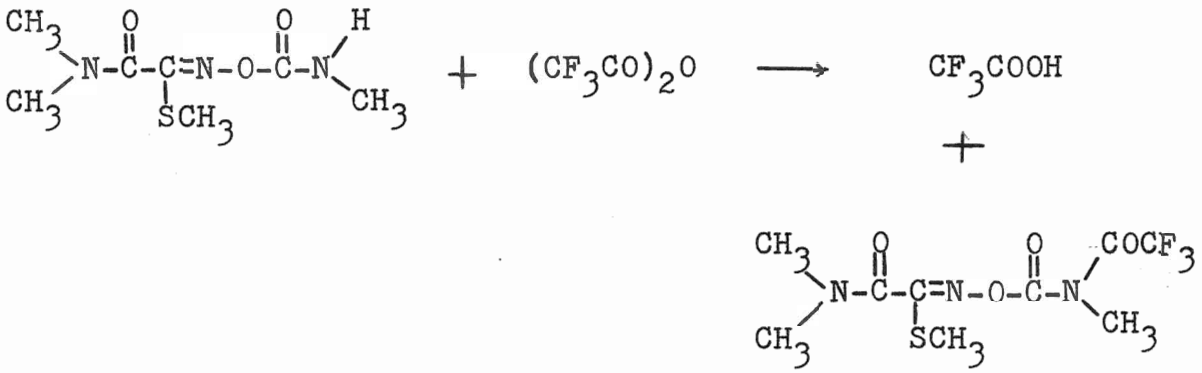


Fig. 12. Chromatograms of oxamyl and oxime obtained on a 3 % XE-60 column


DETECTOR RESPONSE
OXAMYL
OXIME
RETENTION TIME

Because of the carbamate moiety present in the oxamyl molecule, similar reaction will be expected to take place as follow ;



Solutions of oxamyl and corresponding oxime used in this experiment were prepared in ethyl acetate to give concentrations such as 1000 and 100 ppm. The solutions were checked prior to each experiment by running on TLC to ensure that no oxamyl had decomposed. Preliminary investigations did not produce expected results ; various conditions investigated were -- overnight reaction without pyridine at room temperature, overnight reaction with pyridine at room temperature, overnight reaction with pyridine at 6°C in the refrigerator, and reaction at 45°C and for 15 min.

Different solvents namely benzene, ethyl acetate and methanol were also investigated . Ethyl acetate was found to be the most suitable for this study .

Two more factors, length of reaction time and amount of pyridine added , were next investigated. It was at this time that two peaks were found to appear at retention times

0.76 min and 5.7 min consistently. Since the blank samples gave a peak only at retention time 5.7 min , it's most likely that the peak at 0.76 min retention time was due to the derivatized compound. The results from these two experiments are summarized in Table 2 and 3. The GC conditions for these studies were as follow;

column; 90 cm x 3mm (ID) glass column packed with a mixed phase of 11% QF-1 and OV-17 (1:1)

column temperature; 180°C

Injector temperature ; 220°C

Detector temperature; 220°C

From Table 2, it can be assumed that the reaction , trifluoroacetylation, occurs very rapidly . And from Table 3 it was found that 0.01 to 0.1 ml of pyridine is adequate for the completion of the reaction.

The above two derivatives obtained were run on the GC-MS . No further progress , however, was obtained since the detector on this system was less sensitive than the EC detector and did not give any appreciable peak and the mass spectrum did not show any significant peak at m/e 315 which corresponds to the molecular weight of the derivative expected to be produced . Repeated efforts to secure a meaningful result on GC-MS failed. It was decided then that trifluoroacetylation on oxamyl should be abandoned.

III.C.2. Dinitrophenylation

This derivatization method was first introduced by Day et.al. in the determination of microquantities of the Cl

Table 2

Observation of derivatives on chromatogram at different reaction times

Sample	Reaction time (Min)	Retention times of peaks appeared (Min)
Oxamyl	<0.5	0.76, 5.7
	5	0.76, 5.7
	15	0.76, 5.7
	30	0.76, 5.7
	60	0.76, 5.7
Blank		5.7

Note; The reaction was carried out in ethyl acetate and in the presence of pyridine

Table 3

Effect of the amount of pyridine in producing derivatives*

Amount of pyridine (ml)	Retention times of peaks appeared (min)
0.01	0.76, 5.7
0.05	0.76, 5.7
0.1	0.76, 5.7
0.2	0.76, 5.7 , 1.1,1.35,2.1
0.5	0.76, 5.7 , 1.1,1.35,2.1

*The reaction was carried out in ethyl acetate and the reaction times were 15 minutes

to C4 pure amines (46). It involved the reaction of the amines with 2,4-dinitrofluorobenzene to form dinitroaniline derivatives which were then determined by electron-capture gas chromatography. This method was modified and used by Holden et.al.(22), Sumida et.al. (48) and Mendoza et.al.(47) in the determination of carbamate pesticide residues. The extracted carbamate residues were first hydrolysed by alkali, the liberated amines were then reacted with 2,4-dinitrofluorobenzene (DNFB) to form dinitroaniline derivatives and the derivatives formed were analysed by electron-capture gas chromatography.(see Appendix).

Preliminary dinitrophenylation of oxamyl using the buffer described by Sumida et.al. (48) and Mendoza et.al. (47) (5ml of 5% borax solution and 0.5 ml of 1N NaOH or adjusting to pH 10) indicated that both monomethyl and dimethyl amine moieties in the oxamyl molecule were liberated. As a result two peaks due to the two dinitroaniline derivatives , namely, dinitrophenylmethylamine (DNPMA) and dinitrophenyldimethyl amine (DNPDMA) showed up on gas chromatograms. For oxime, since it has only one amine moiety, only one dinitroaniline derivative ,dinitrophenyldimethylamine (DNPDMA)was obtained.

To obtain the best separation , different liquid phases such as 5 % QF-1, 3 % SE-30, DC-200 and 3 % XE-60 were investigated. Of these , only 3 % XE-60 gave good resolution for the two derivatives. The GC conditions for the best resolution were as follow--

Column ; 90 cm x 3 mm ID glass column packed with 3 % XE-60

on gaschrom Q 80/100 mesh

Column temperature ; 190°C

Injector temperature ; 220°C

Detector temperature ; 220°C

Carrier gas (nitrogen) flow rate ; 40 ml/min

Under these conditions , the retention times of DNPDMA and DNPMA were 1.06 min and 1.34 min respectively. These were checked and confirmed by injecting pure DNPDMA and DNPMA.

III.C.2.(a). Base hydrolysis and dinitrophenylation

The effects of pH of the buffer solutions were next investigated . A set of buffer solutions ranging from pH 7.8 to 12 was prepared. These were then used in the dinitrophenylation of oxamyl , oxime , dimethylamine hydrochloride and methylamine hydrochloride solutions. The GLC results were summarized in Table 4 and shown in Fig. 13,14, and 15.

In order to investigate the effects of pH in a wider range, the experiment was repeated with the inclusion of buffers of pH 8.2 and 13 . The GLC results were summarized in Table 5 , Figs. 16 and 17 .(Dimethylamine HCl and methylamine HCl were not included because of less importance)

From these two sets of data, it is obvious that the yield of DNPMA was reasonably stable and did not fluctuate much between pH 8.2 and 12 . The yields of DNPDMA, however , varied a great deal for oxamyl , oxime and dimethylamine.HCl. The drop in yield at pH 11 was especially noticeable when the adjacent

Table 4

The effects of pH of the buffer solutions on dinitrophenylation

pH	Detector response of derivatives (peak height in cm)					
	oxamyl		oxime	dimethylamine HCl	methylamine.HCl	
	DNPDMA	DNPMA	DNPDMA	DNPDMA	DNPMA	
7.8	D*	4.4	D*	13.0	4.9	
8.88	3.6	8.8	D*	16.6	12.6	
10.0	13.8	8.5	7.65	23.3	9.9	
11.0	4.9	8.9	1.8	14.3	8.4	
12.0	17.1	7.9	13.3	18.9	11.4	

D* = decomposed ; shoulder peak appeared

Fig. 13. The effects of pH of the buffer solutions on dinitrophenylation of oxamyl

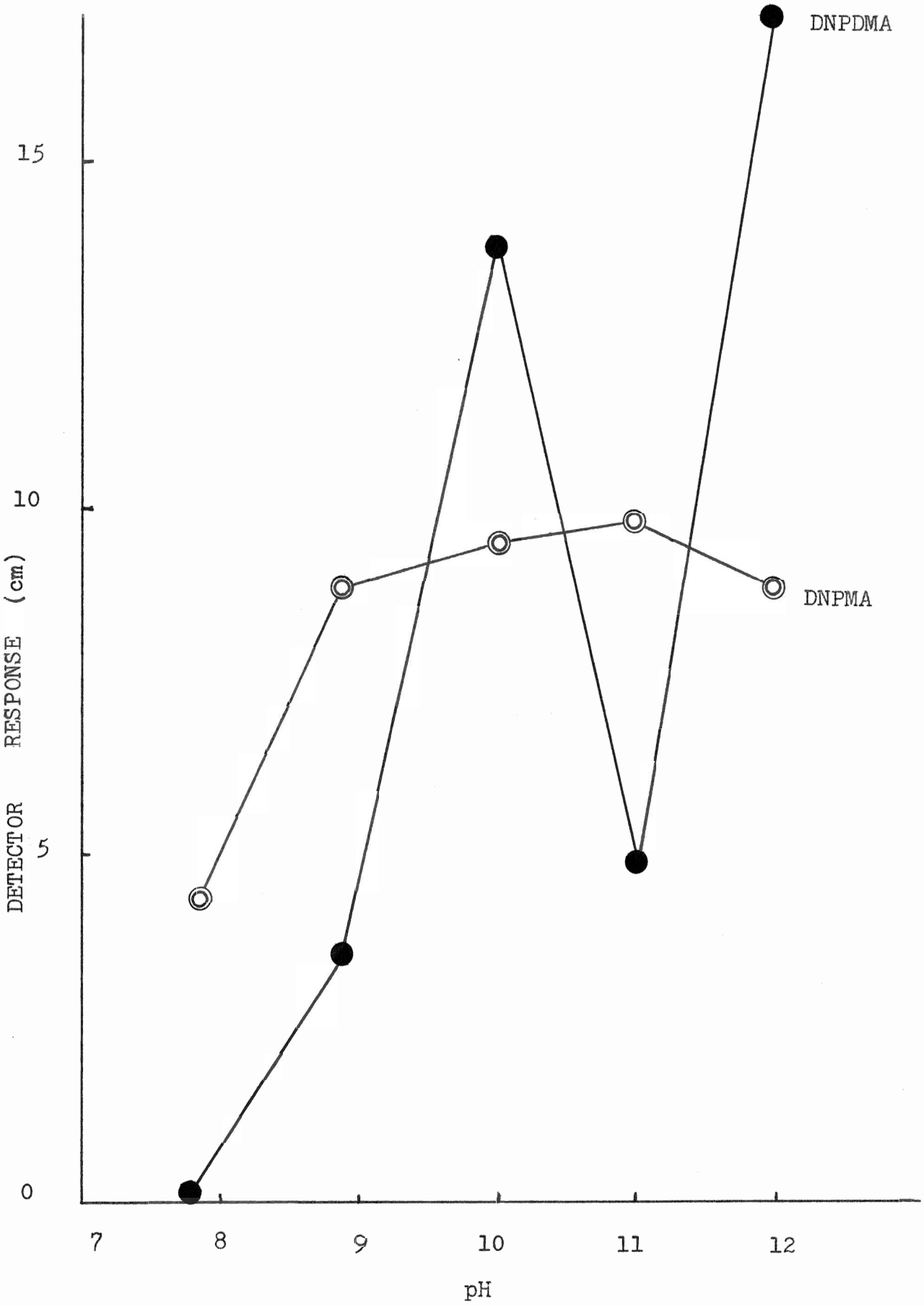

DNPDMA
DNPMA
15
10
5
0
DETECTOR RESPONSE (cm)
7
8
9
10
11
12
pH

Fig. 14. The effects of pH of the buffer solutions on dinitrophenylation of oxime

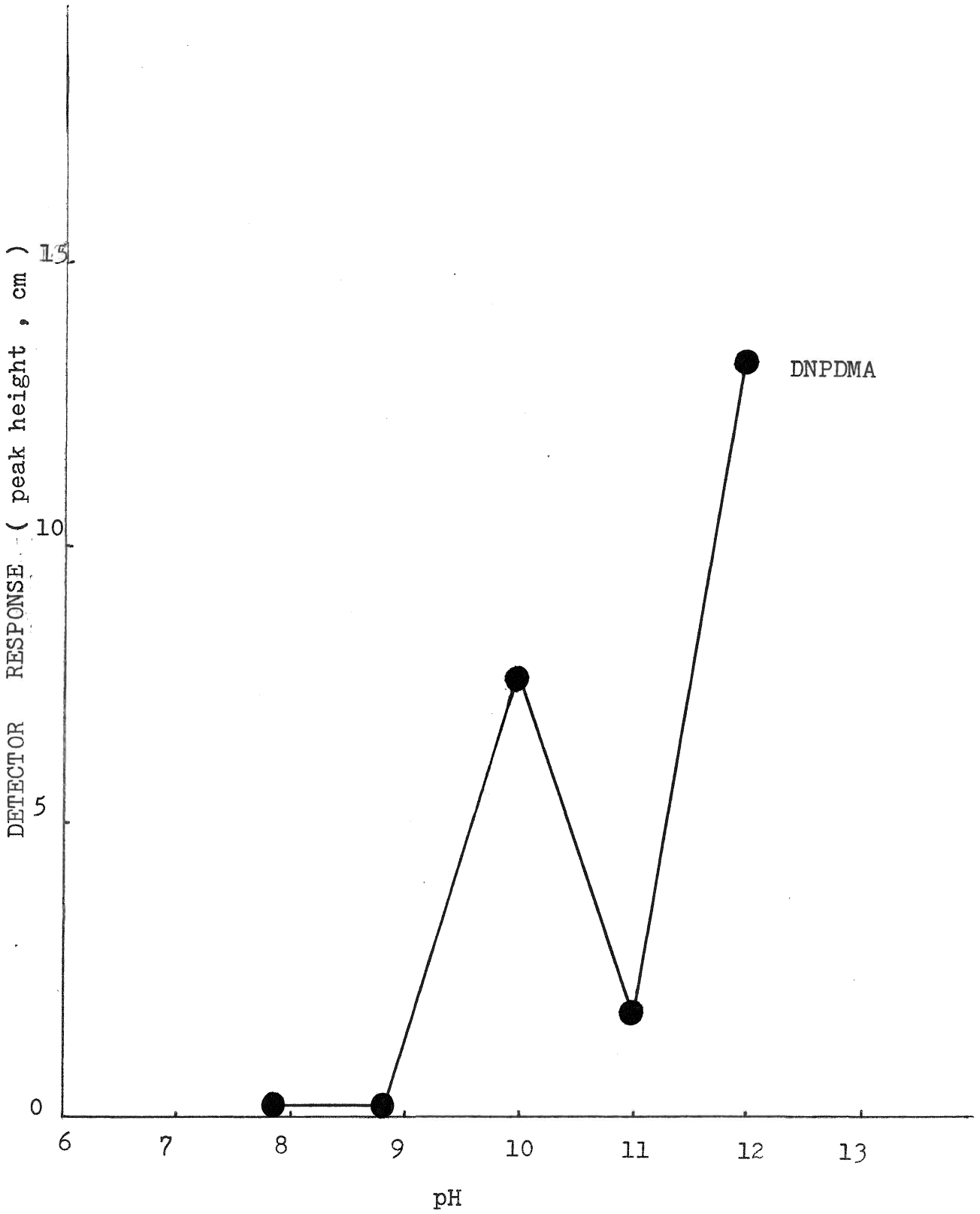

DETECTOR RESPONSE (peak height , cm)
15
10
5
0
DNPDMA
6
7
8
9
10
11
12
13
pH

Fig. 15. The effects of pH of the buffer solutions on dinitrophenylation of methylamine hydrochloride and dimethylamine hydrochloride

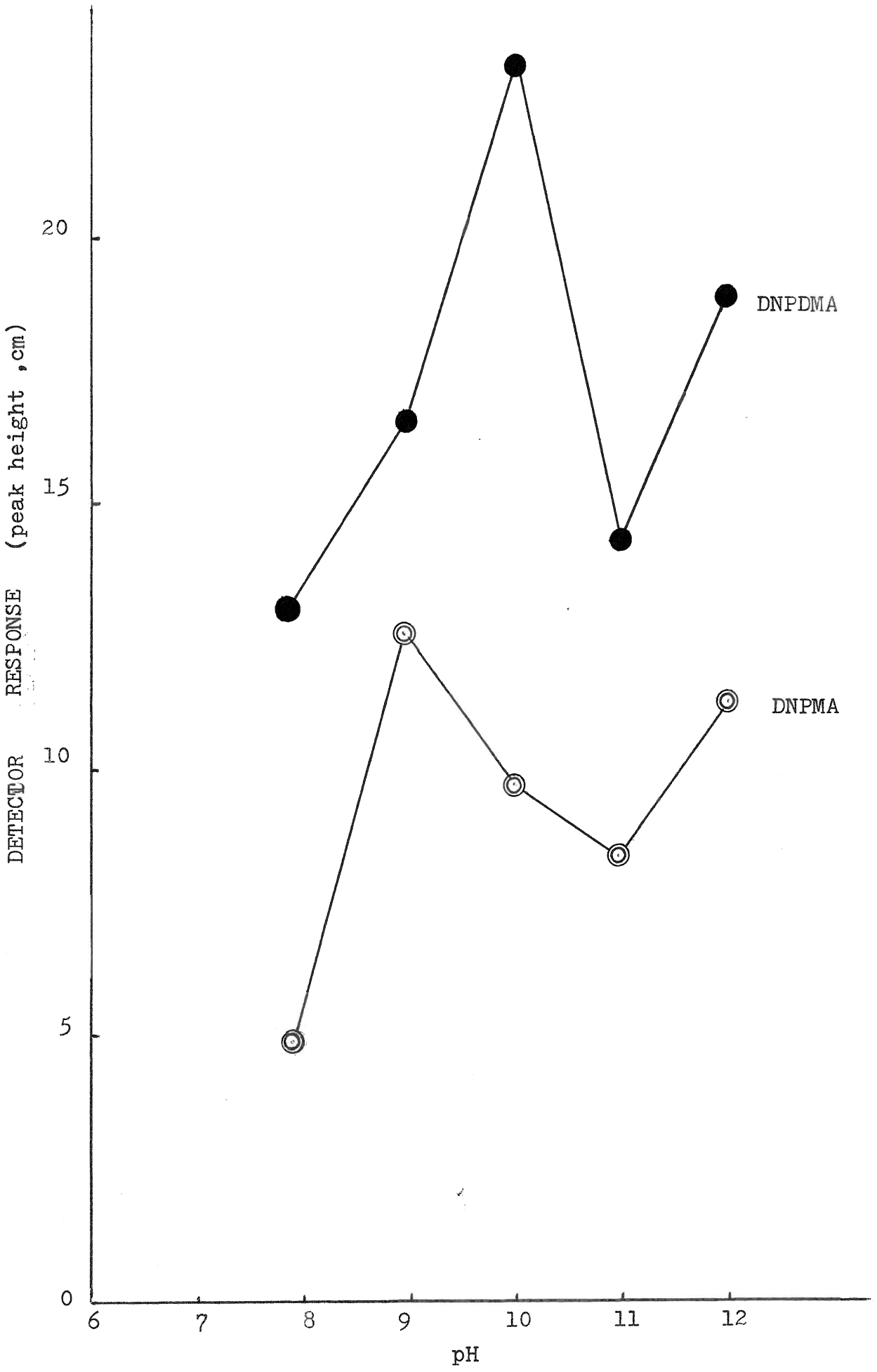

DETECTOR RESPONSE (peak height, cm)
20
15
10
5
0
6
7
8
9
10
11
12
pH
DNPDMA
DNPMA

Table 5

The effects of pH of the buffer solutions on dinitrophenylation of oxamyl and oxime

pH	Detector response of derivatives (peak height in cm)		
	oxamyl		oxime
	DNPDMA	DNPMA	DNPDMA
7.8	D*	9.0	D*
8.2	D*	13.5	D*
8.88	3.2	14.6	4.7
10.0	24.4	15.8	26.0
11.0	5.2	14.4	3.6
12.0	29.8	16.5	29.0
13.0	14.0	5.9	17.0

D* = decomposed ; shoulder peak appeared

Fig. 16. The effects of pH of the buffer solutions on dinitrophenylation of oxamyl (including pH 13)

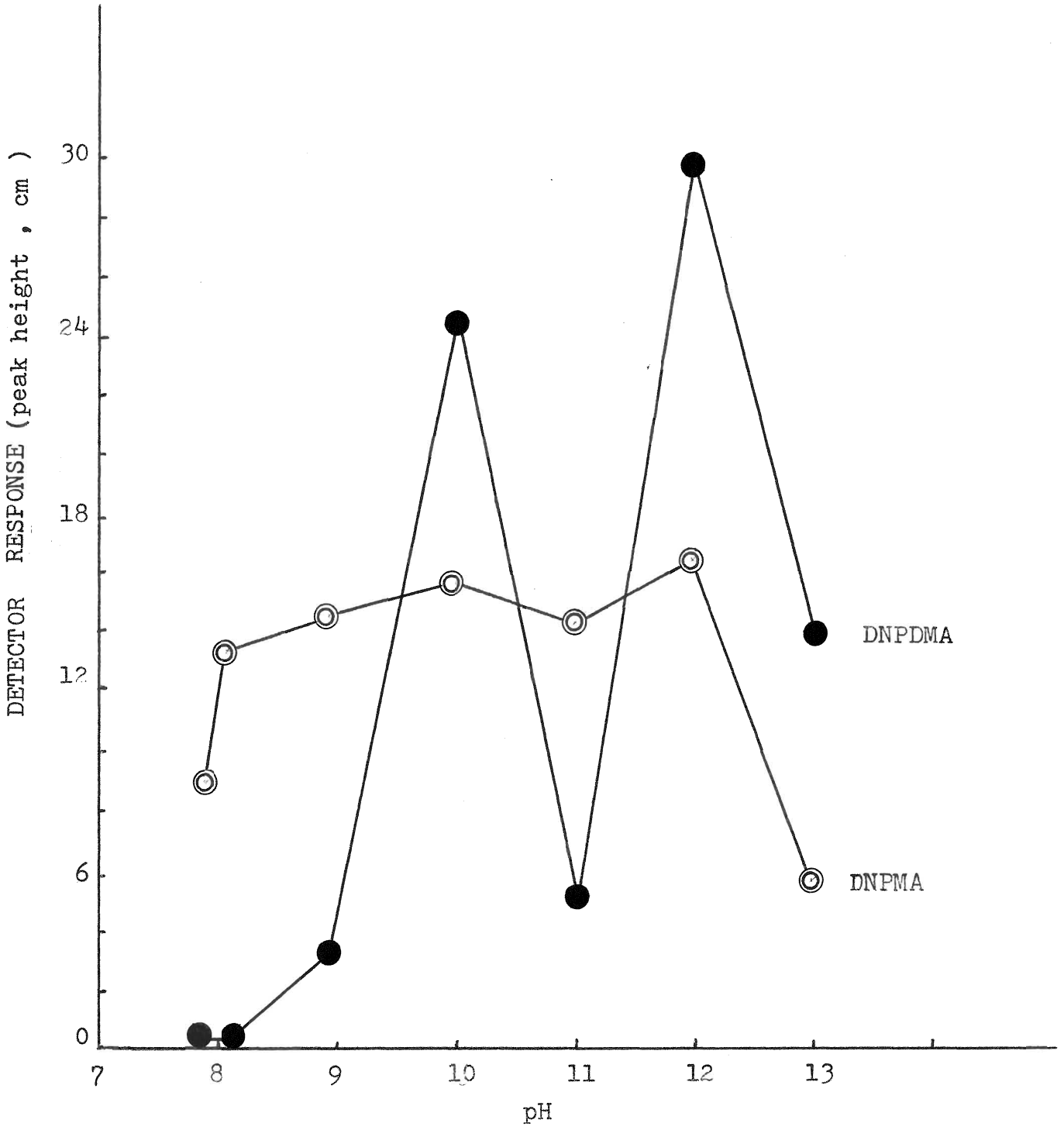
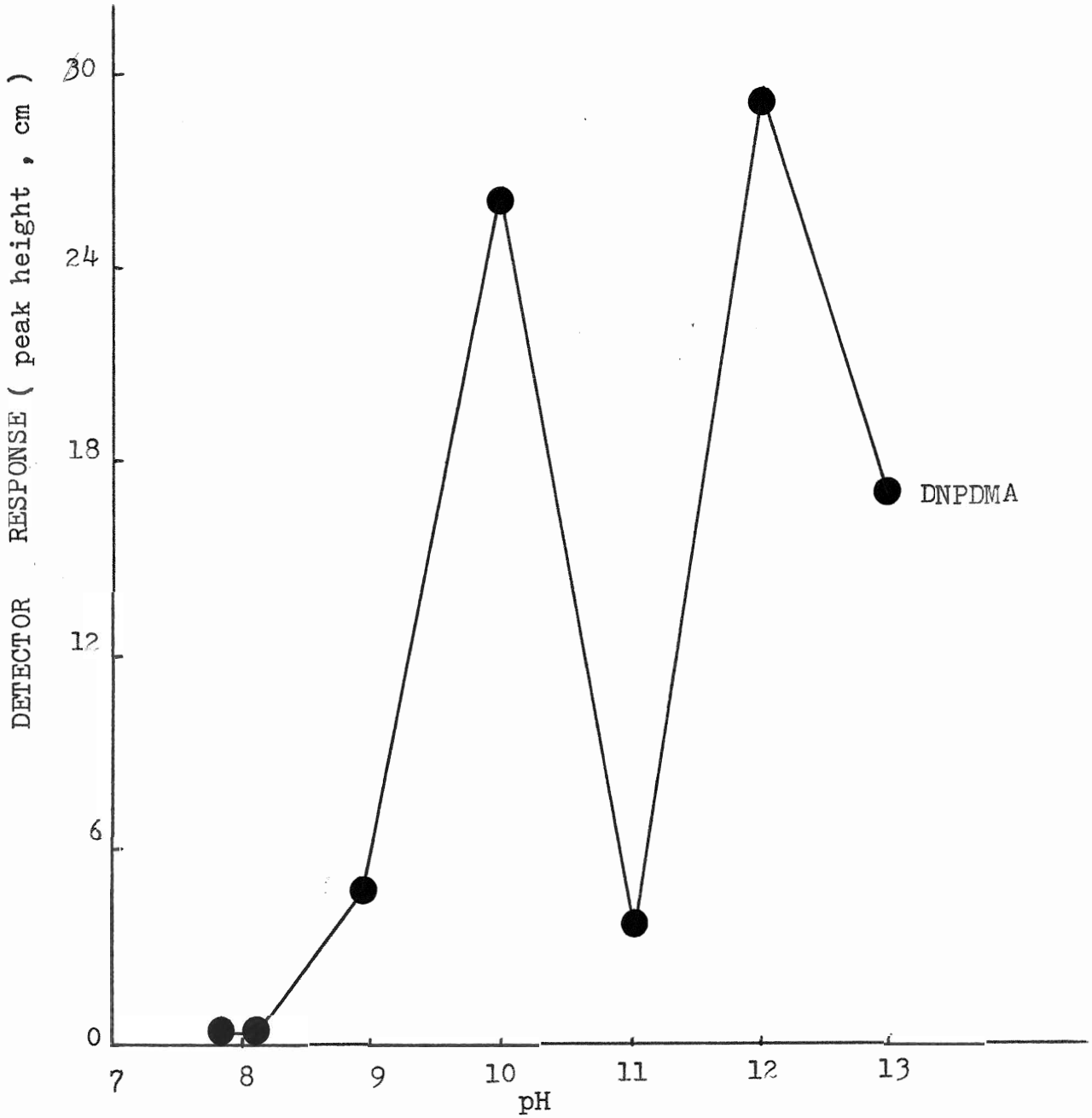

DETECTOR RESPONSE (peak height , cm)
30
24
18
12
6
0
7
8
9
10
11
12
13
pH
DNPDMA
DNPMA

Fig. 17. The effects of pH of the buffer solutions on dinitrophenylation of oxime (including pH 13)


DETECTOR RESPONSE (peak height , cm)
30
24
18
12
6
0
7
8
9
10
11
12
13
pH
DNPDMA

pH 10 and pH 12 gave much higher yields . An investigation of pH at each reaction step from pH 10 to 13 revealed that the buffer capacity of pH 11 (Na_2HPO_4 and NaOH) was very weak. From Table 6 , it was apparent that at pH 11, the drop of pH from the first step (after adding buffer) to the second step (after adding DNFB) was much more significant that at other pH's. A new buffer of pH 11 (0.05 N $NaHCO_3$ and 0.1 N NaOH) was next investigated. A measure of pH at different reaction step indicated the drop of pH between the first step and the second step was less significant than the old pH 11 (Table 7). Subsequently , the yield of DNPDMA was much higher as shown in Table 8 and on Fig.18.

From Fig. 18 , it can be concluded that the yield of DNPMA was maximum between pH 8.8 and pH 12, and for DNPDMA , the maximum yield will be obtained at pH 12. DNPDMA was found to be decomposed at pH 7.8 and 8.2 for both oxamyl and oxime when extra peaks appeared on the shoulders.

The effect of reaction temperature was next investigated. The optimum reaction temperature was found to be around 80°C for both oxamyl and oximino compound ,as shown in Table 9 and on Fig. 19 .

The length of reaction time and hydrolysis time were next optimized . In all cases , the same length of time was applied for both hydrolysis and reaction and the time was set at 5 ,10 , 15 , and 20 minutes . The results were summarized

Table 6

Change of pH at different reaction steps for buffer of pH 10 to pH 13

pH of buffer	sample	after adding buffer	after adding DNFB (reagent)	after adding glycine
10	dimethylamine HCl	9.87	9.22	7.89
	blank	9.86	9.30	7.90
11	oxamyl	10.98	8.13	7.38
	blank	10.98	8.15	7.37
12	oxamyl	11.88	11.24	7.99
	blank	11.91	11.30	7.98
13	oxime	12.65	12.59	8.90
	blank	12.67	12.67	8.94

Table 7

Change of pH at different reaction steps for new buffer of pH 11

pH	sample	after adding buffer	after adding DNFB	after adding glycine
11	oxamyl	11.03	9.37	7.75
	oxime	11.06	9.36	7.77
	blank	11.12	9.39	7.71

Table 8

The effect of pH of the buffer solutions on dinitrophenylation of oxamyl (new pH 11.0)

pH	Detector response (peak height in cm) oxamyl	
	DNPDMA	DNPMA
7.8	D*	9.1
8.2	D*	13.45
8.88	3.2	14.5
10.0	24.35	15.65
11.0	25.55	17.6
12.0	29.8	16.5
13.0	14.1	5.85

D* = decomposed ; shoulder peak appeared

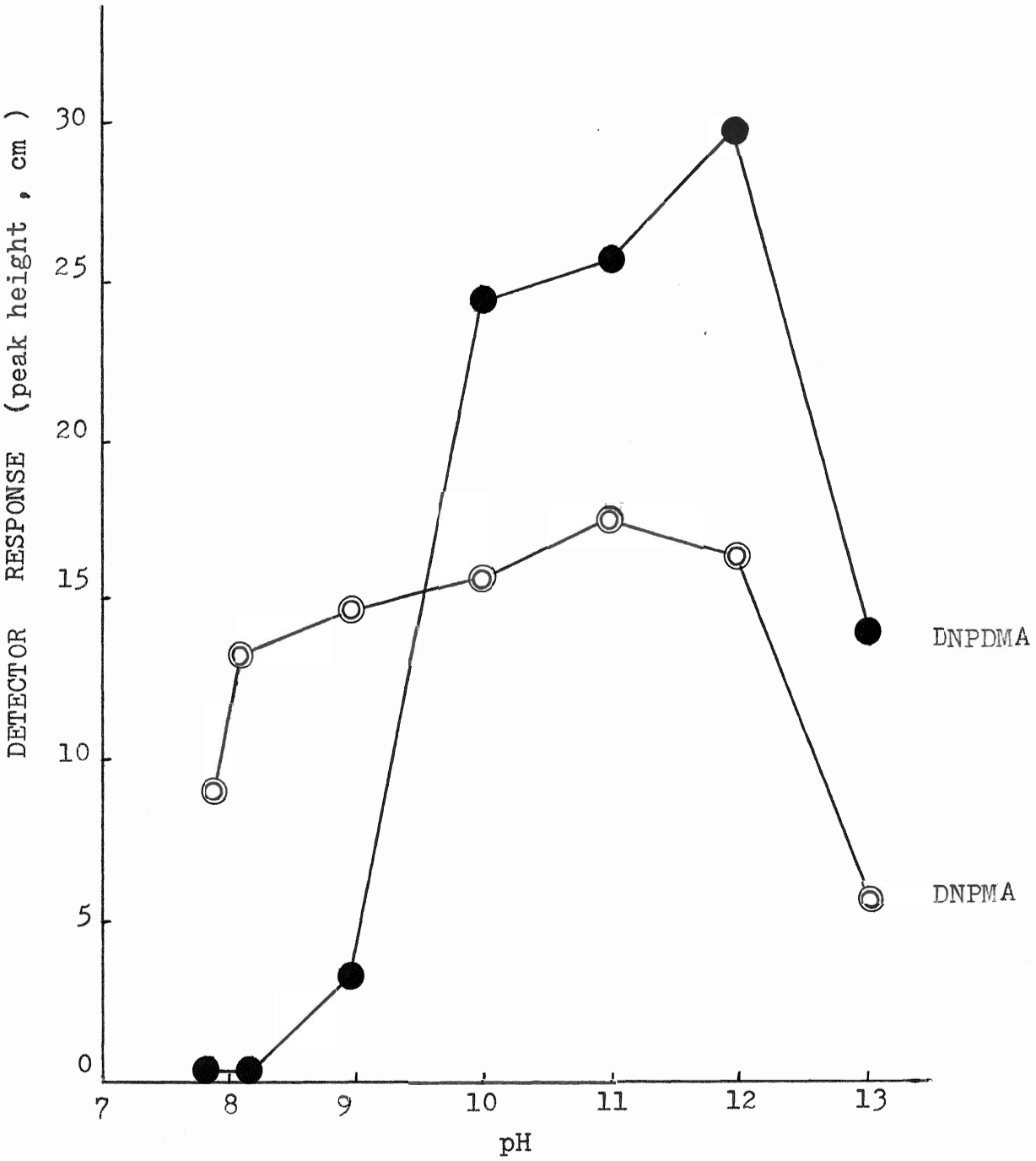

DETECTOR RESPONSE (peak height , cm)
30
25
20
15
10
5
0
7
8
9
10
11
12
13
pH
DNPDMA
DNPMA

Fig. 18. The effects of pH of the buffer solutions on dinitrophenylation of oxamyl (new pH 11.0)

Table 9

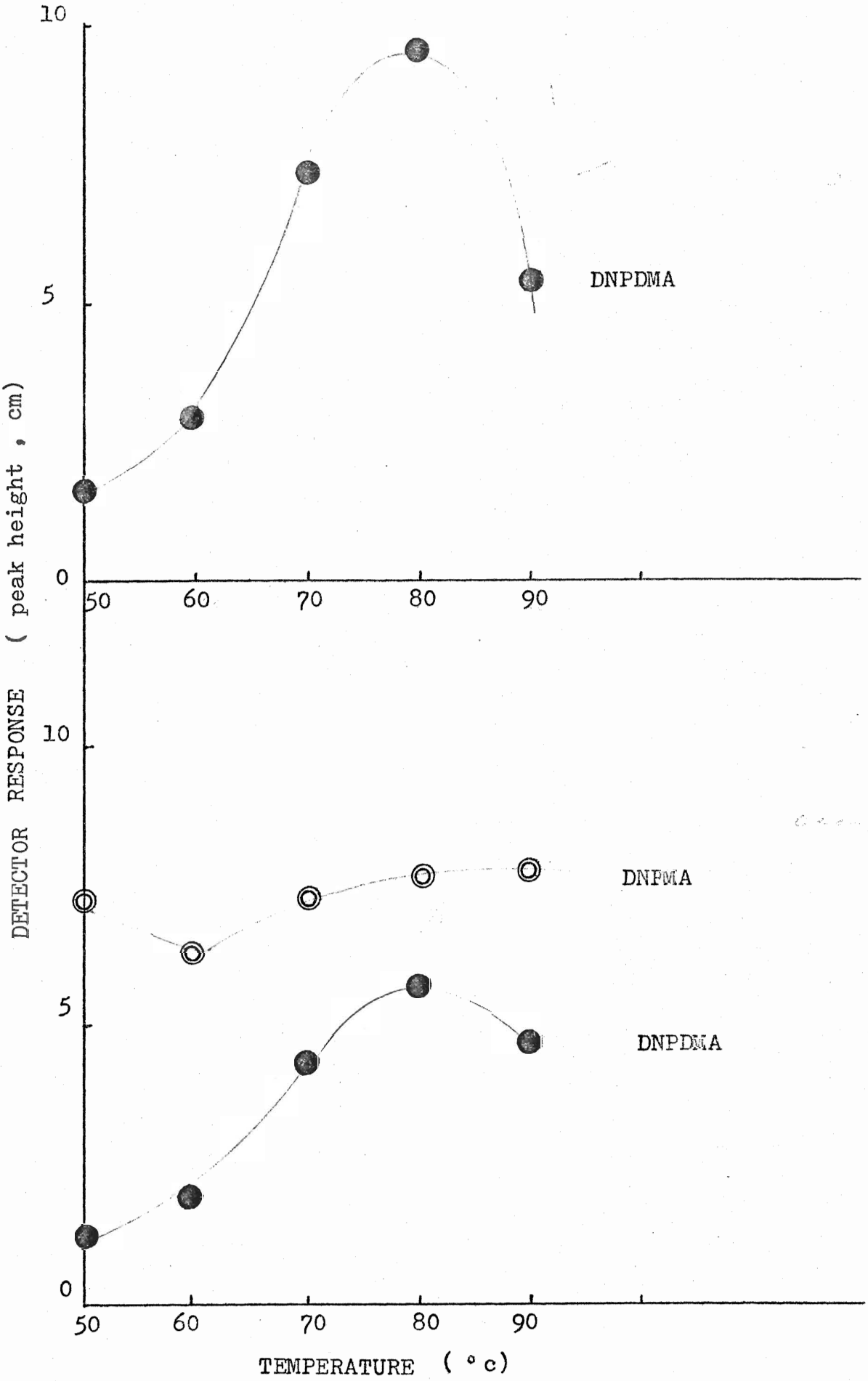
Effect of reaction temperature on the formation of dinitrophenyl derivatives

Temperature	Detector response [a] (peak height in cm)		
	oxamyl		oxime
	DNPDMA	DNPMA	DNPDMA
50°C	1.2 d	7.30	1.7d
60°C	1.9 d	6.45	3.0d
70°C	4.30	7.45	7.40
80°C	5.70	7.85	9.60
90°C	4.70	8.00	5.4 d

a = average of two measurements

d = slightly decomposed ; shoulder peak appeared

Fig. 19. The effects of reaction temperature


10
5
0
DETECTOR RESPONSE (peak height , cm)
50
60
70
80
90
DNPDMA
10
5
0
DNPMA
DNPDMA
50
60
70
80
90
TEMPERATURE (°c)

in Table 10. The results of varying the reaction time while keeping hydrolysis time at 10 min , or varying hydrolysis time while keeping reaction time at 10 min would be discussed later when attentions will be focused on DNPMA only .

From Table 10 , it is obvious that the optimum time period for the formation of DNPMA is at 10 minute.

The next factor investigated was the choice of base. Instead of NaOH , KOH was used . But the yields of DNPMA and DNPDMA were not altered.

The percentage yields of DNPMA and DNPDMA from oxamyl and oxime were then calculated based on different experiments done on different days . The data are tabulated in Table 11 .

The yields of DNPMA ranged from 80.1 % to 120 % (Table 11) . This range was judged to be acceptable though variation seemed noticeable .The yields of DNPDMA , however, was low and not reproducible . As our main objective was to develop a method that can determine both oxamyl and oxime simultaneously , a better yield of DNPDMA would be desirable. Since base hydrolysis failed to improve this yield , acid hydrolysis was investigated .

III.C.2.(b). Acid hydrolysis and dinitrophenylation

Hydrolysis time was first investigated . As shown in Table 12 , the GC response for DNPDMA increased significantly as the hydrolysis time extended ; the maximum response was obtained after overnight hydrolysis . The response for DNPMA,

Table 10

Time factor on the formation of dinitrophenylamine derivatives

Time (min)		Detector response[a] (peak height in cm)		
Hydrolysis	Reaction	oxamyl		oxime
		DNPDMA	DNPMA	DNPDMA
5	5	1.7	3.7	2.2
10	10	2.6	4.6	2.5
15	15	2.1	4.5	2.5
20	20	2.2	4.4	3.1

a = average of two measurements

Table 11

Reproducibility in the

percentage yield of DNPDMA and DNPMA from oxamyl and oxime on different days

Day	Yield, %		
	from oxamyl[a]		from oxime[b]
	DNPDMA	DNPMA	DNPDMA
1	16.8	102.5	24.4
2	43.7	120.0	42.6
3	21.1	80.1	24.0
4	48.7	102.5	48.0

a = 1 ml of looo ppm oxamyl solution was used.

b = 1 ml of looo ppm oxime solution was used.

Table 12

Detector response(expressed in peak height, cm) for DNPDMA and DNPMA formed from oxamyl and oxime after a variable period of acid hydrolysis

Hydrolysis time (min)	Detector response[a] (peak height in cm)		
	oxamyl		oxime
	DNPDMA	DNPMA	DNPDMA
10	1.3	2.1	0.0
20	1.5	1.4	0.0
30	2.2	2.2	< 1
60	2.0	1.5	2.2
120	4.9	1.6	6.5
overnight	8.3	1.7	19.7

a = average of two measurements

however , was less dependent on hydrolysis time .

The percent yield of the overnight samples of both oxamyl and oxime were calculated based on a standard DNPDMA curve . The yields were 39.5 % and 53.0 % for oxamyl and oxime respectively . Other conditions such as increasing the temperature to 95°C and 105°C , using 1N sulfuric acid or glacial acetic acid instead of concentrated sulfuric acid , and different ways of adding reagent dinitrofluorobenzene such as dissolving it in dioxane or in benzene . These efforts , however , failed to produce any better yield.

Although it was more advantageous if a method can determine both oxamyl and oxime residues simultaneously, the determination of oxime is not particularly important because it is non-toxic (39). Besides , since a GLC method is available for the oxime (38) all attentions thereafter, were focused on base hydrolysis and on the determination of DNPMA .

Previously , the amount of dinitrofluorobenzene (DNFB) added was 1 % in dioxane (10 µl in 1 ml dioxane) .It was of interest to find out the optimum level of DNFB in the reaction with oxamyl . The amount of DNFB was varied from 1 µl to 25 µl in 1ml dioxane . The results can be seen on Table 13 . The maximum response was obtained when the concentration of DNFB was 10 µl in 1 ml dioxane .

The last factor investigated to obtain a good yield of DNPMA was the hydrolysis time and reaction time. The

Table 13

Detector response of DNPMA in relation to different concentrations of DNFB

Concentration of DNFB solution in dioxane[a] (%)	Detector response (peak height, cm) of DNPMA
0.1	0.0
0.5	3.6
1.0	10.3
2.0	9.6
2.5	7.5

a= The constant volume of 1 ml of each solution was taken.

reaction time was varied from 5 min to 30 min while keeping hydrolysis time at 10 min and vice versa . The detector response of DNPMA formed from oxamyl in relation to hydrolysis time and reaction time is summarized in Table 14.

From the results , it was judged that the best hydrolysis time and reaction time is 10 minutes for both. Since the significance of all factors involved in this DNPMA formation reaction and the optimum reaction conditions had been successfully obtained , the linearity and lowest detection limit of DNPMA formed from oxamyl was investigated.

Oxamyl aqueous solutions ranging from 0.5 µg/ml to 8 µg/ml were prepared. One ml each of these solutions was hydrolysed and derivatized to DNPMA . Since the solutions at the time of injection into the GC were one hundredth time diluted , the concentrations became 0.005 ppm to 0.08 ppm (expressed in oxamyl concentrations) . The whole experiment was repeated two more times and the reproducibility of the results was satisfactory (Table 15).

The relationship between the amounts of chemical injected (expressed as the original weight of oxamyl) and the average detector responses is shown in Fig. 20 . The line is linear from 0.02 ng to 0.32 ng , indicating that oxamyl can be detected at the level of around 0.5 µg/ml in a solution at the time of reaction by this derivatization method and the detection is linear from 0.5 µg/ml to at least 8 µg/ml .

Table 14

Detector response [a] (expressed in peak height , cm) of DNPMA formed from oxamyl in relation to hydrolysis time and reaction time

Hydrolysis time (min)	Reaction time (min)	Peak height (cm)
5	10	15.3
10	10	15.4
15	10	15.4
20	10	14.6
30	10	11.6
10	5	13.0
10	10	13.3
10	15	12.1
10	20	12.2
10	30	12.6

a = average of two measurements

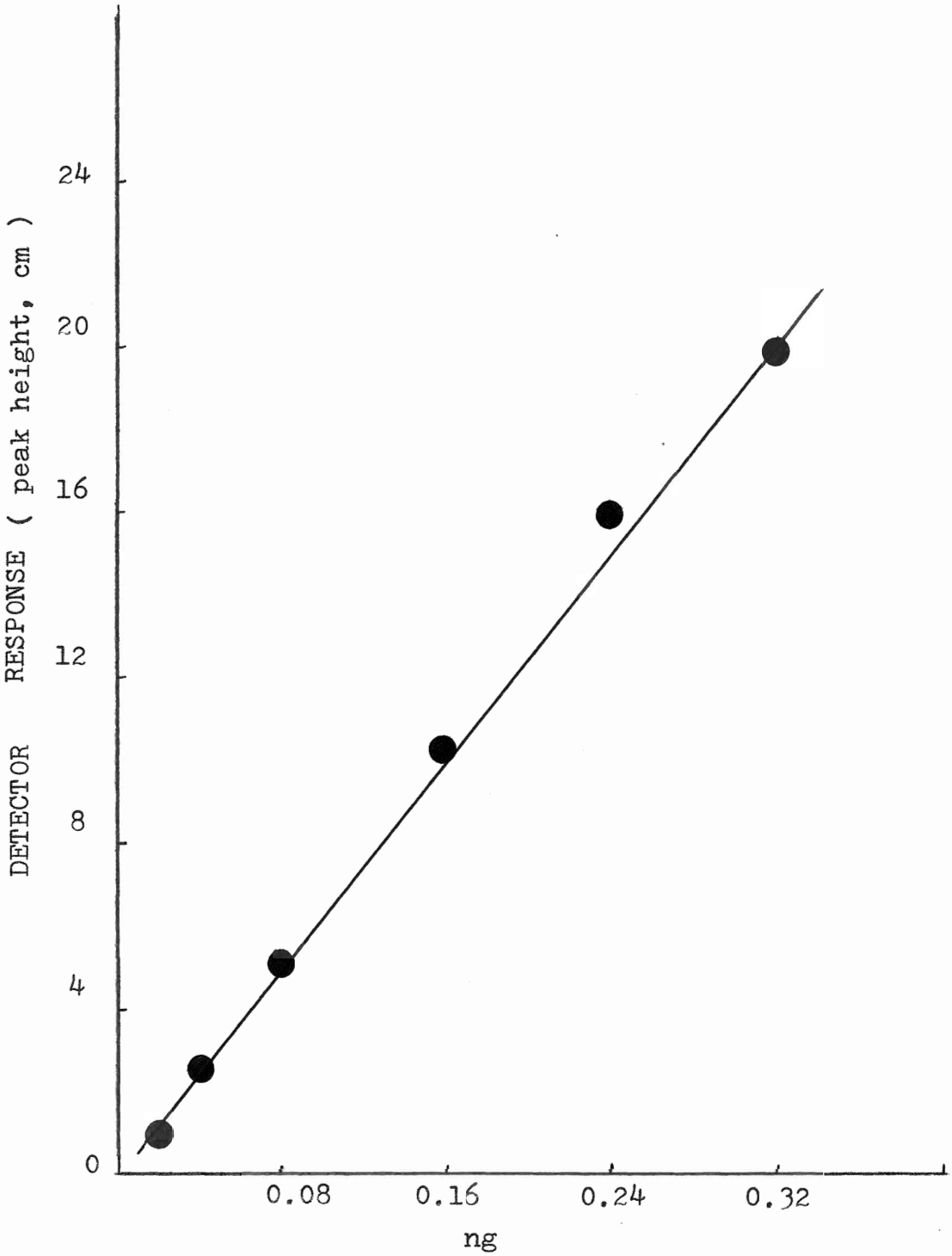
Table 15

Linearity and reproducibility of detector responses[a] from three experiments

Starting concentrations of oxamyl (ppm)	Amounts injected (expressed as oxamyl , ng)	Expt.1	Expt.2	Expt.3	Average (of 1,2, and 3)
0.5	0.02	0.9	0.9	1.0	0.9
1.0	0.04	2.4	2.5	2.5	2.5
2.0	0.08	5.1	5.1	5.0	5.1
4.0	0.16	10.5	10.1	10.0	10.2
6.0	0.24	16.2	16.1	15.7	16.0
8.0	0.32	19.7	19.9	19.7	19.7

a = average of two measurements

Fig. 20. Linearity and lowest detection limit of oxamyl


DETECTOR RESPONSE (peak height, cm)
24
20
16
12
8
4
0
0.08
0.16
0.24
0.32
ng

The amounts of DNPMA (and thus the percentage yields) corresponding to the peak heights for each level of concentrations in Table 16 were obtained from a standard curve such as Fig.21. The percent yields for each oxamyl concentration level are summarized in Table 17. With the exception of 0.5 ppm starting concentration of oxamyl, the percent yields at all other concentration ranges 1.0 to 8.0 ppm were fairly consistant being at the average of 74.8 % .

III. D. Dinitrophenylation of Oxamyl on Tobacco Leaves

III.D.1. Dinitrophenylation of oxamyl which was added to tobacco leaves

Two varieties of tobacco leaves were investigated. Harrow-vel , grown in the green house of the Research Station, Agriculture Canada , Vineland Station , was first used. After derivatization , both the spiked and blank leaf samples gave a DNPMA peak on gas chromatogram . This was suspected to be due to pirimicarb used in the control of aphids in the green houses. (pirimicarb is known to degrade photochemically to a compound containing a monoamine moiety (52)). For this reason , the second variety obtained from Delhi Research Station , Delhi-34 was spiked with oxamyl and investigated . (Delhi-34 tobacco leaves were sprayed with only an organophosphorus insecticide). It was suprising, however, to find a small DNPMA peak in the blank leaf sample. This interference could be traced back to

Table 16

Detector response [a](peak height) of standard DNPMA in relation to its concentrations

Concentrations of DNPMA (ppm)	Amount injected (volume of injection=4 µl)	Peak height (cm)
0.005	0.02	2.2
0.01	0.04	4.7
0.02	0.08	7.4
0.04	0.16	15.2
0.06	0.24	21.3
0.08	0.32	27.6

a = average of two measurements

Fig. 21. Standard curve of DNPMA

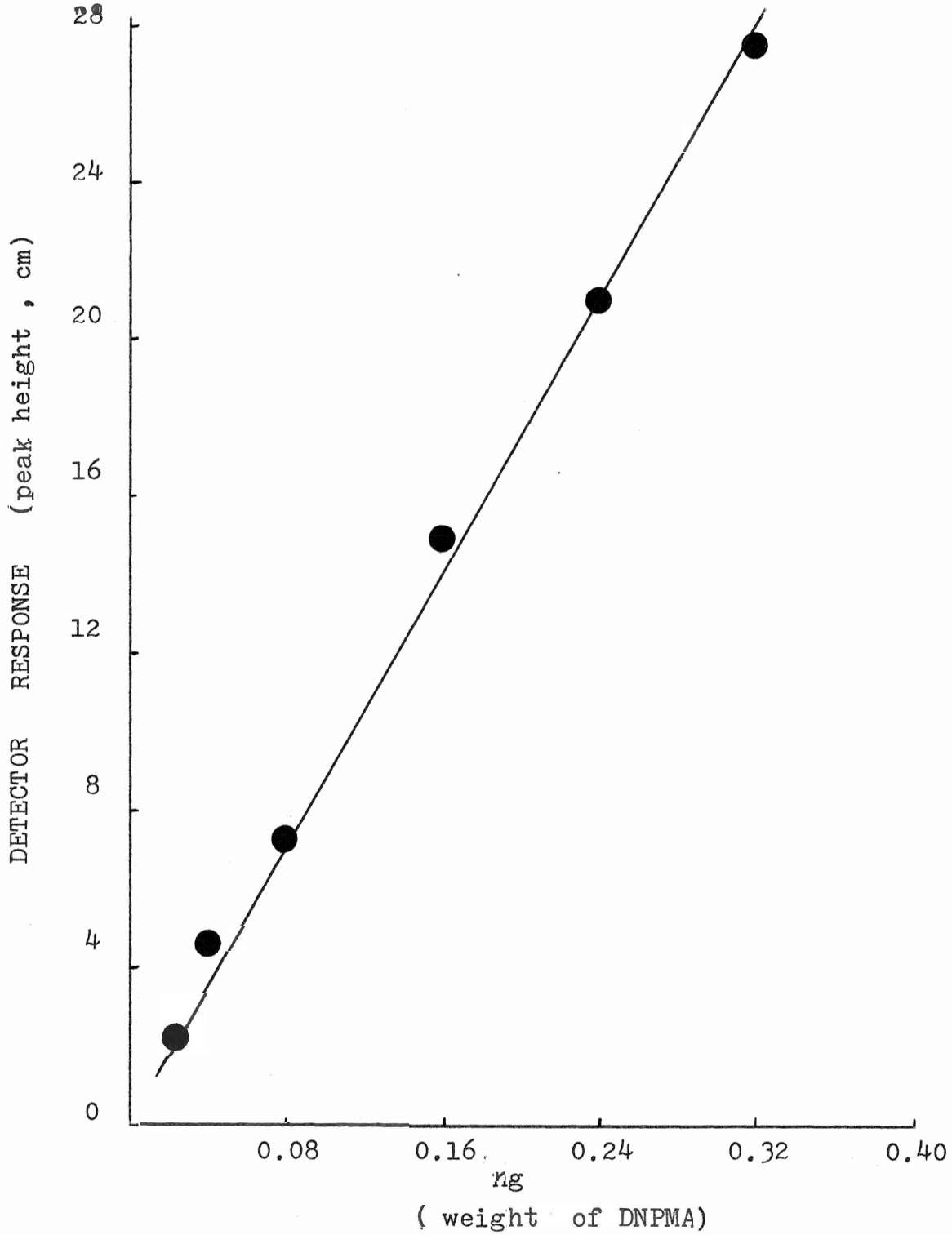

DETECTOR RESPONSE (peak height , cm)
28
24
20
16
12
8
4
0
0.08
0.16
0.24
0.32
0.40
Mg
(weight of DNPMA)

Table 17

Percent yields of DNPMA from oxamyl of different concentrations

Starting oxamyl concentration (ppm)	Expected weight of DNPMA (ng)	Actual weight of DNPMA obtained (ng)	Yield, %
0.5	0.018	0.008	44.5
1.0	0.036	0.026	72.3
2.0	0.072	0.052	72.3
4.0	0.143	0.107	74.4
6.0	0.216	0.174	80.6
8.0	0.288	0.214	74.3

its origin in the natural components of tobacco leaves. The previous assumption that pirimicarb caused the interference in Harrow-vel tobacco leaves was found to be in valid because non-sprayed blank tobacco leaves also gave a DNPMA peak . In addition, pirimicarb is known to have a short half-life (52). The fact that Delhi-34 variety gave a smaller DNPMA peak than Harrow-vel variety indicated that the former had a smaller quantity of interfering material (see Fig. 22). Re peated efforts to clean up the interfering impurity by washing with ethyl acetate , benzene etc.were not successful. Therefore , correction was made on the peak height measurements for oxamyl spiked samples by subtracting peak height values of the blank samples .

III.D.1.(a). Clean up of leaf extracts by thin layer chromatography

This topic is best discussed in combination with the next paragraph.

III.D.1.(b). Gas liquid chromatography

The GC chromatograms obtained on extracted leaf samples gave a few extra peaks before and after the DNPMA peak. In order to remove these extra peaks, the samples were first cleaned up by TLC . After developing, the spots corresponding to the DNPMA spot were cut off and extracted with benzene . The recoveries of the clean up process were found to be qualitative (see Table 18).

Previously, in III.C.2., an experiment was run to determine the linearity and the lowest detectable limit of the

Fig.22. Chromatograms of Harrow-vel and Delhi-34 blank tobacco leaves after derivatization

(a) = Delhi-34

(b) = Harrow-vel

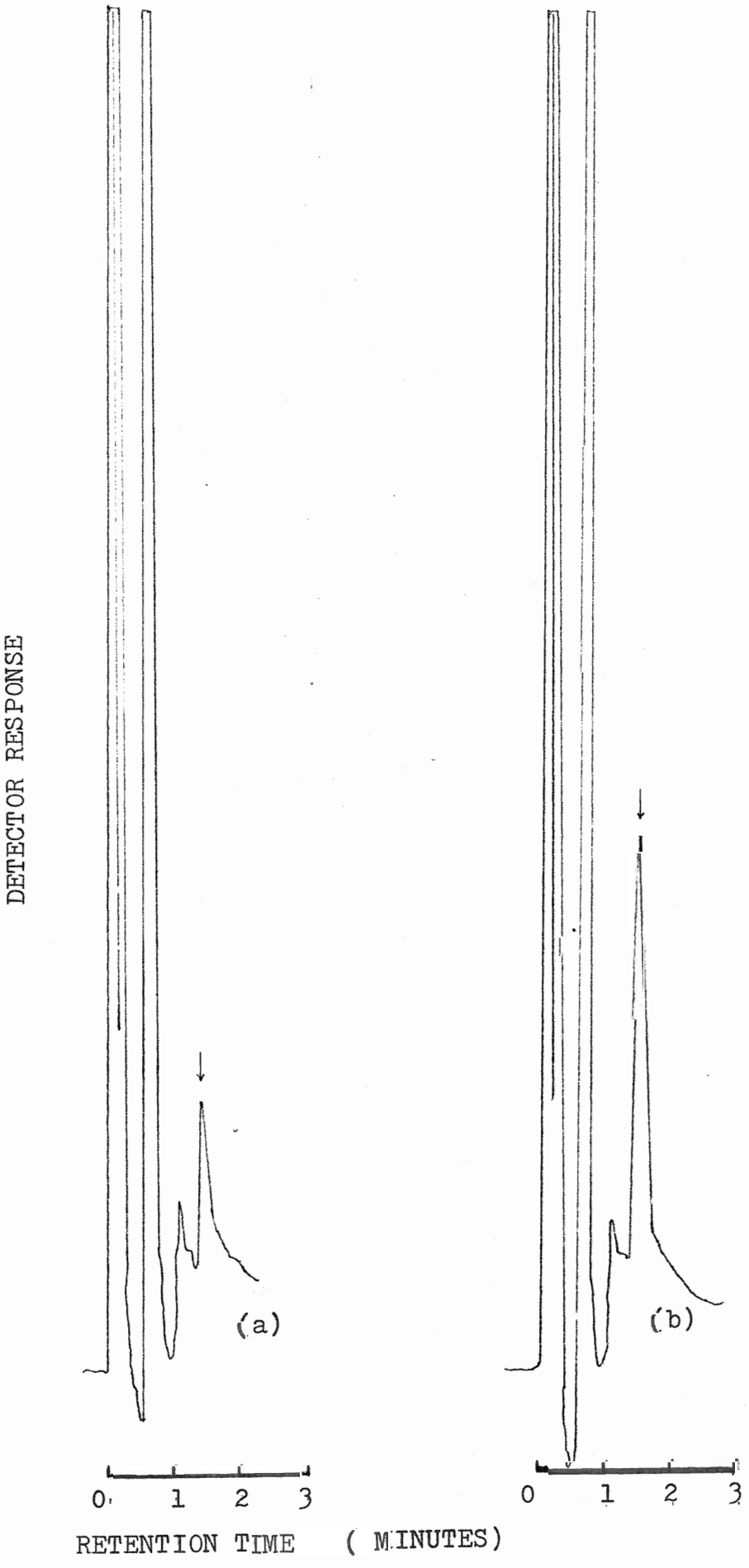

DETECTOR RESPONSE
(a)
(b)
0
1
2
3
0
1
2
3
RETENTION TIME (MINUTES)

Table 18

Gas chromatographic response of DNPMA before and after TLC clean up

Sample	Detector response[a] (peak height in cm)	
	Before TLC	After TLC
Blank	1.0	1.0
0.2 ppm oxamyl standard	2.5	2.7
0.2 ppm oxamyl spiked leaves	3.7	3.7
2.0 ppm oxamyl standard	26.2	26.5

a = corrected for blank

pure derivative DNPMA . The experiment was repeated and the standard curve obtained was used as reference to the curves obtained with the following experiments. Aqueous solutions of standard oxamyl (concentration range of 0.1 to 2.0 ppm) were processed through the complete procedures of extraction, derivatization , clean up and GLC , with or without leaf samples. The detector response thus obtained are tabulated in Tables 19, 20,and 21, and those results are summarized in Fig. 23. Note that Fig.23 was obtained by plotting peak heights against the actual weights of oxamyl or DNPMA injected . As shown in Fig. 23, the plotted lines are reasonably linear. The detection limit of oxamyl spiked samples could be as low as 0.1 ppm, a level sensitive enough for pesticide residue analysis.

III.D.2. Determination of oxamyl in field tobacco samples

Field tobacco samples were obtained from one of Agriculture Canada experimental farms in Jordan Station , ontario. The samples were stored at -15°C in a storage room, research station, Agriculture Canada, Vineland Station. These plants were transplanted in the soil treated with oxamyl (at 7 lb active ingredient/acre) and about one month later , plants were collected and stored in the storage room for future use. They were mainly used for research in the control of nematodes by a scientist specializing in nematology . Two of these samples were analysed for oxamyl residues. The results are summarized

Table 19

Detector response[a] of DNPMA standards

Concentration of DNPMA (ppm)	Actual weight of DNPMA injected (ng)	Peak height (cm)
0.01	0.03	2.4
0.02	0.06	4.1
0.05	0.15	10.4
0.10	0.30	18.7
0.20	0.60	41.0

a = an average of two measurements

Table 20

Detector response[a] of DNPMA formed from standard oxamyl solutions

Concentrations of oxamyl standard solutions (ppm)	Actual weight injected (expressed as oxamyl) (ng)	Peak height (cm)
0.1	0.03	1.7
0.2	0.06	3.4
1.0	0.30	17.0
2.0	0.60	33.0

a = an average of two measurements

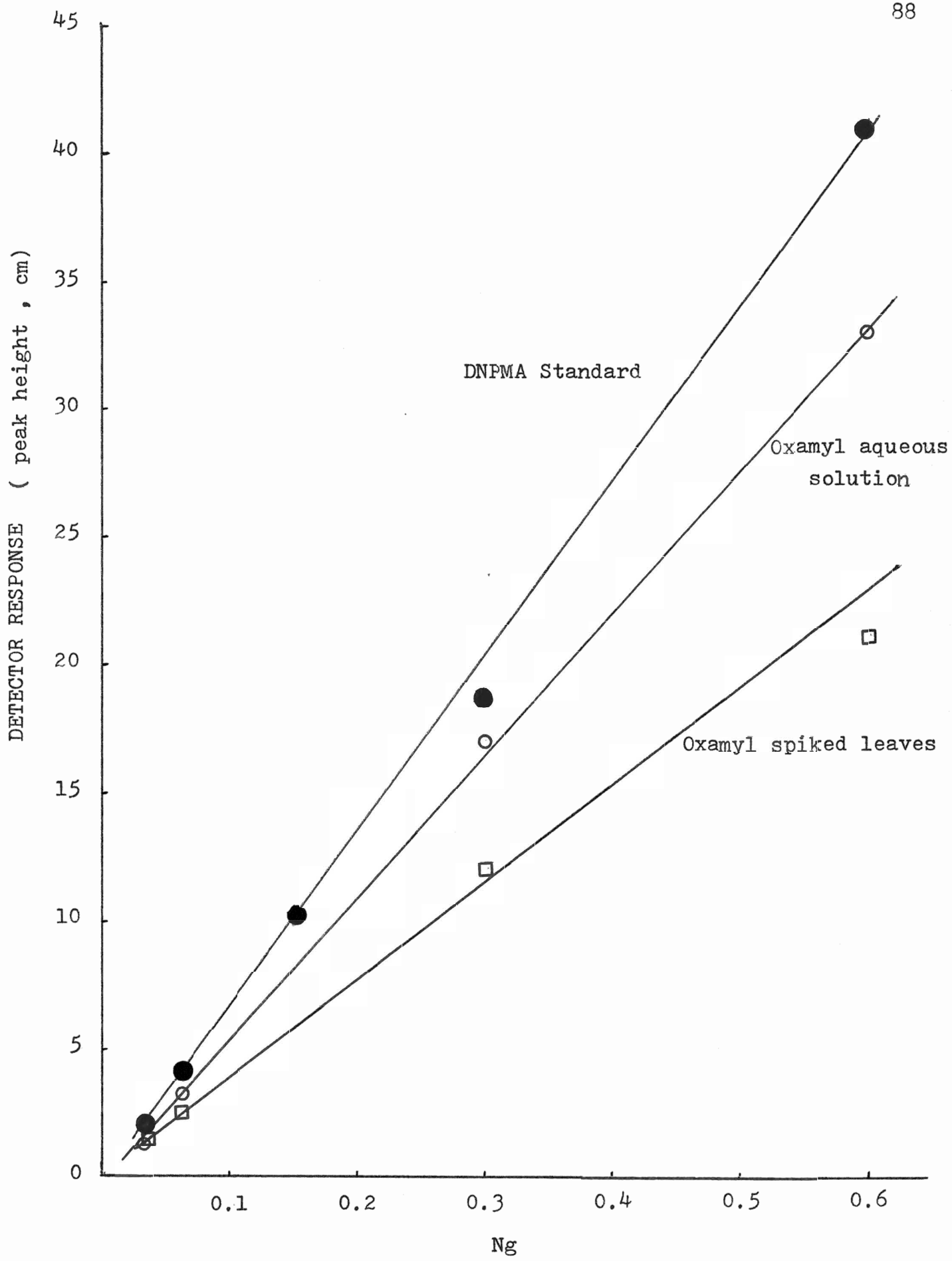
Table 21

Detector response [a] of DNPMA formed from tobacco leaves spiked with standard oxamyl solutions

Concentration of oxamyl standard solutions (ppm)	Actual weight injected (expressed as oxamyl) (ng)	Peak height (cm)
0.1	0.03	1.8
0.2	0.06	2.3
1.0	0.30	12.0
2.0	0.60	21.0

a = corrected for blank and an average of two measurements

Fig.23. Linearity and lowest detection limit for oxamyl spiked leaves samples


45
40
35
30
25
20
15
10
5
0
DETECTOR RESPONSE (peak height , cm)
DNPMA Standard
Oxamyl aqueous solution
Oxamyl spiked leaves
0.1
0.2
0.3
0.4
0.5
0.6
Ng

in Table 22. Chromatograms of one of the samples and a blank are shown in Fig. 24.

After correcting for the blank , the oxamyl residue levels were found to be 0.61 and 0.59 ppm. It was also noted that interference from field tobacco leaves was more prominent than the interference found with the green house leaf samples .

III.D.3. Determination of oxamyl in field soil samples

Four of the field soil samples were analysed for oxamyl residues . Prior to extraction , the moisture content of soil samples was adjusted to about 40 % to improve the extraction efficiency because the moisture content of those samples originally was very low being around 1 % . It was reported that extraction of pesticides was incomplete when the soil was either too dry or too wet (53). Chiba and Morley reported that a moisture content of between 25 and 50 % is ideal for extraction (53).

Four samples (Nos.1 ,2 , 3, and 4) were analysed and the results are summarized in Table 23. The chromatograms of a blank and sample No.2 are shown in Fig. 25.

It was found , as expected that interference from soil samples was significantly less than that from tobacco leaves. In the blank samples, no DNPMA peak appeared on the chromatogram , and accordingly, no correction was necessary for the blanks.

Table 22

Residues of oxamyl in field tobacco leaves

Sample	Concentration[a] in tobacco leaves (ppm)
1	0.61
2	0.59

a = corrected for blanks

Fig. 24. Chromatograms of a field tobacco leaf samples treated with oxamyl and of a blank

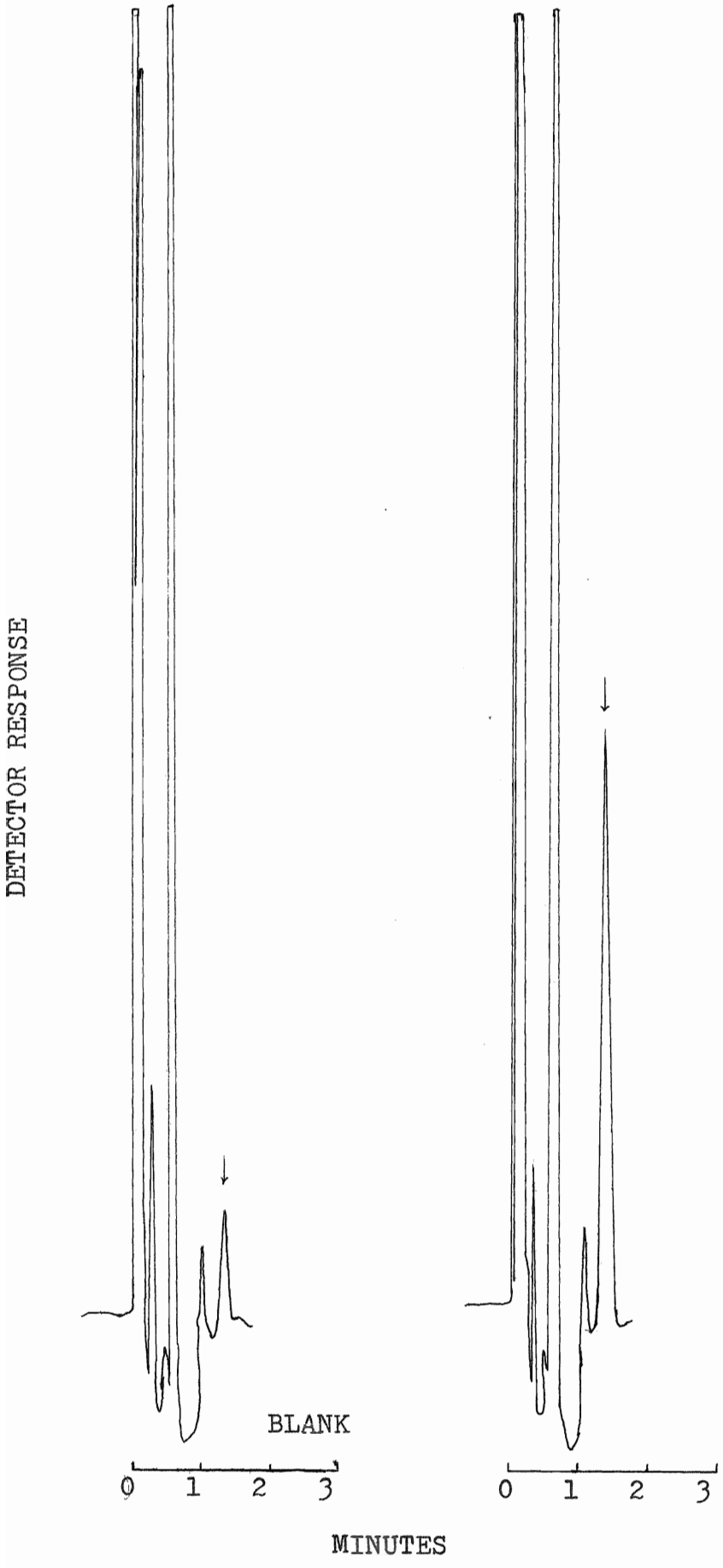

DETECTOR RESPONSE
BLANK
0
1
2
3
0
1
2
3
MINUTES

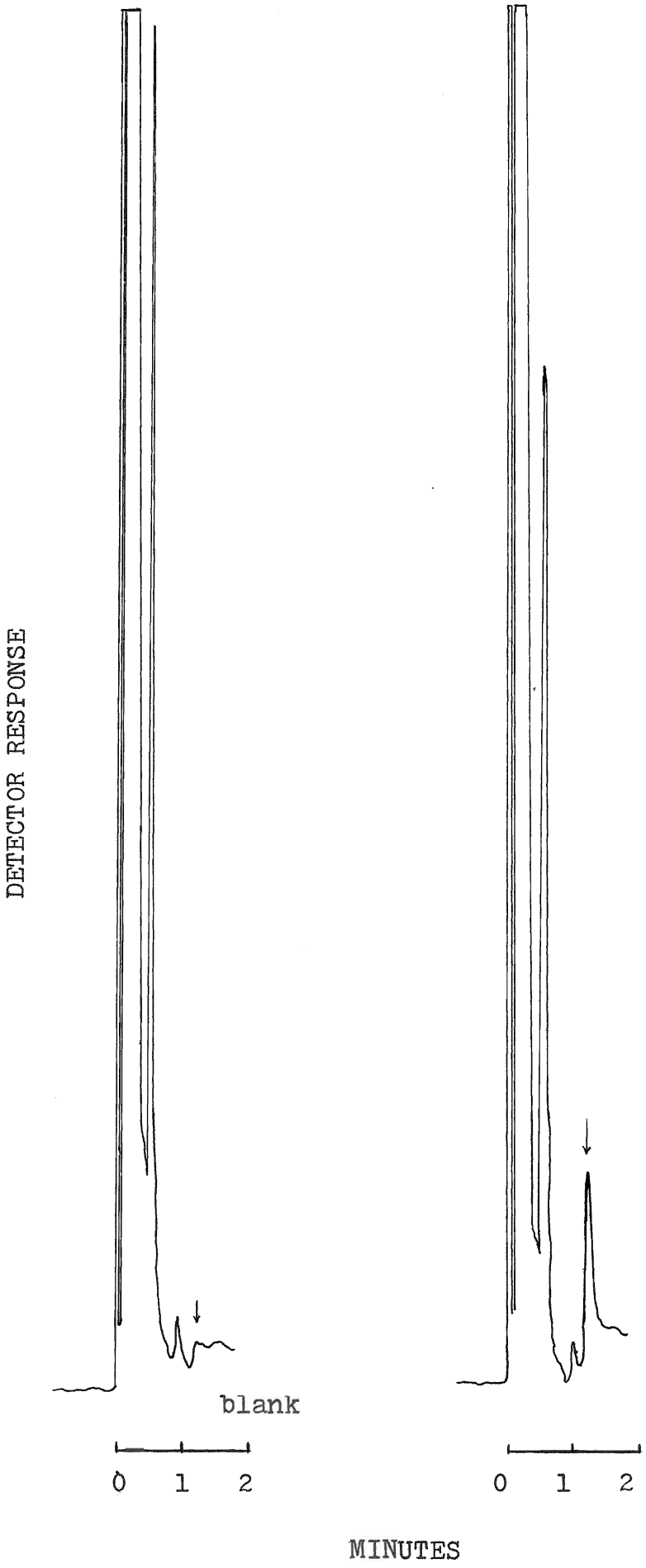
Table 23

Oxamyl residues in field soil samples

Sample	Concentration[a] (ppm)
1	0.22
2	0.14
3	0.23
4	0.21

a = based on air-dried weight of 50 gm samples

Fig. 25. Chromatograms of a field soil sample treated with oxamyl and of a blank


DETECTOR RESPONSE
blank
0
1
2
0
1
2
MINUTES

IV. SUMMARY AND CONCLUSION

During the course of this work , a new GLC-EC method for the determination of oxamyl was developed . This method was found to be applicable for the determination of oxamyl residues in both tobacco leaves and soil samples .

The basic principle of this method is to produce a derivative which is highly sensitive to an electron capture detector. The derivative described is dinitrophenylmethylamine, DNPMA,which is produced from monomethylamine ; a base hydrolysis product of oxamyl and dinitrofluorobenzene ,DNFB. Experimental conditions such as pH, reaction temperature, reaction time, amount of reagent added were thoroughly investigated and optimized. It was necessary for the sake of saving time in further determination procedures and better appearance of the chromatogram, to clean up the final product by TLC technique. A temperature programmer will also be able to cut down the time for each injection.

During the course of this work , it was also discovered that oxamyl and its degradation product , oximino compound , could be separated by TLC using ethyl acetate as a developing solvent. A similar attempt was made by De Wildt but he failed to separate these compounds because his selection of solvent system was unsatisfactory (54). The TLC separation of ^{14}C -labeled oxamyl and corresponding

oximino compound was later confirmed by Harvey,Jr. (50) of Du Pont . It was also by TLC that oxamyl was found to degrade at room temperature to oximino compound in benzene solution after an extended period of time . Oxamyl was also found to decompose to its main degradation product on GLC column ; this finding prompted the development of this derivatization method of analysis.

At the beginning of this work , the purity and structures of oxamyl and oxime obtained from Du Pont were verified by utilizing NMR and mass spectrometry . An attempt was also made to explain the fragmentation patterns of some major peaks in the mass spectra of oxamyl and oxime .

It would be of interest to apply this derivatization method for the determination of oxamyl residues in other commodities . One significant advantage of the present method is its simplicity and rapidity . It would be of the greatest value when a large number of samples are to be analysed routinely.

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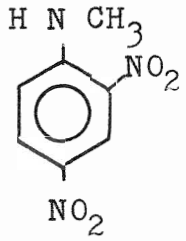
APPENDIX

The formation of DNPDMA and DNPMA from carbamates;

carbamates (oxamyl or oxime) —hydrolysis→ $MeNH_2$ + Me_2NH (for oxamyl)

carbamates (oxamyl or oxime) —hydrolysis→ Me_2NH (for oxime)

$MeNH_2$ —DNFB→  (DNPMA)

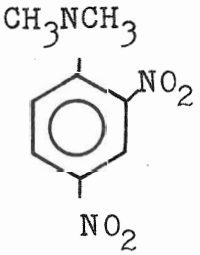
Me_2NH —DNFB→  (DNPDMA)

Fig. 26. Mass spectrum of DNPDMA

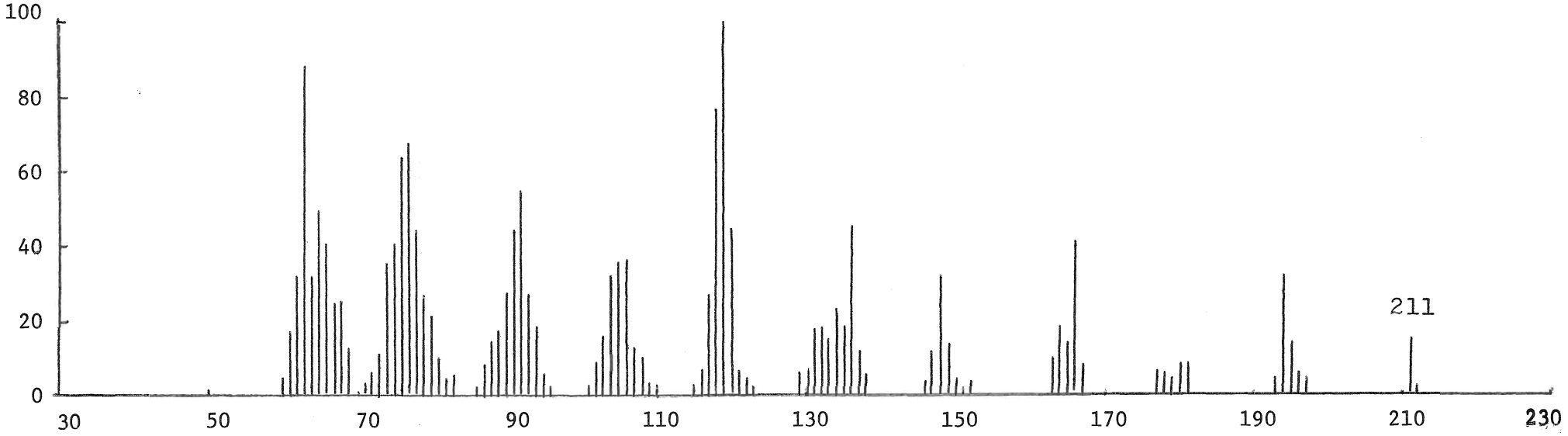
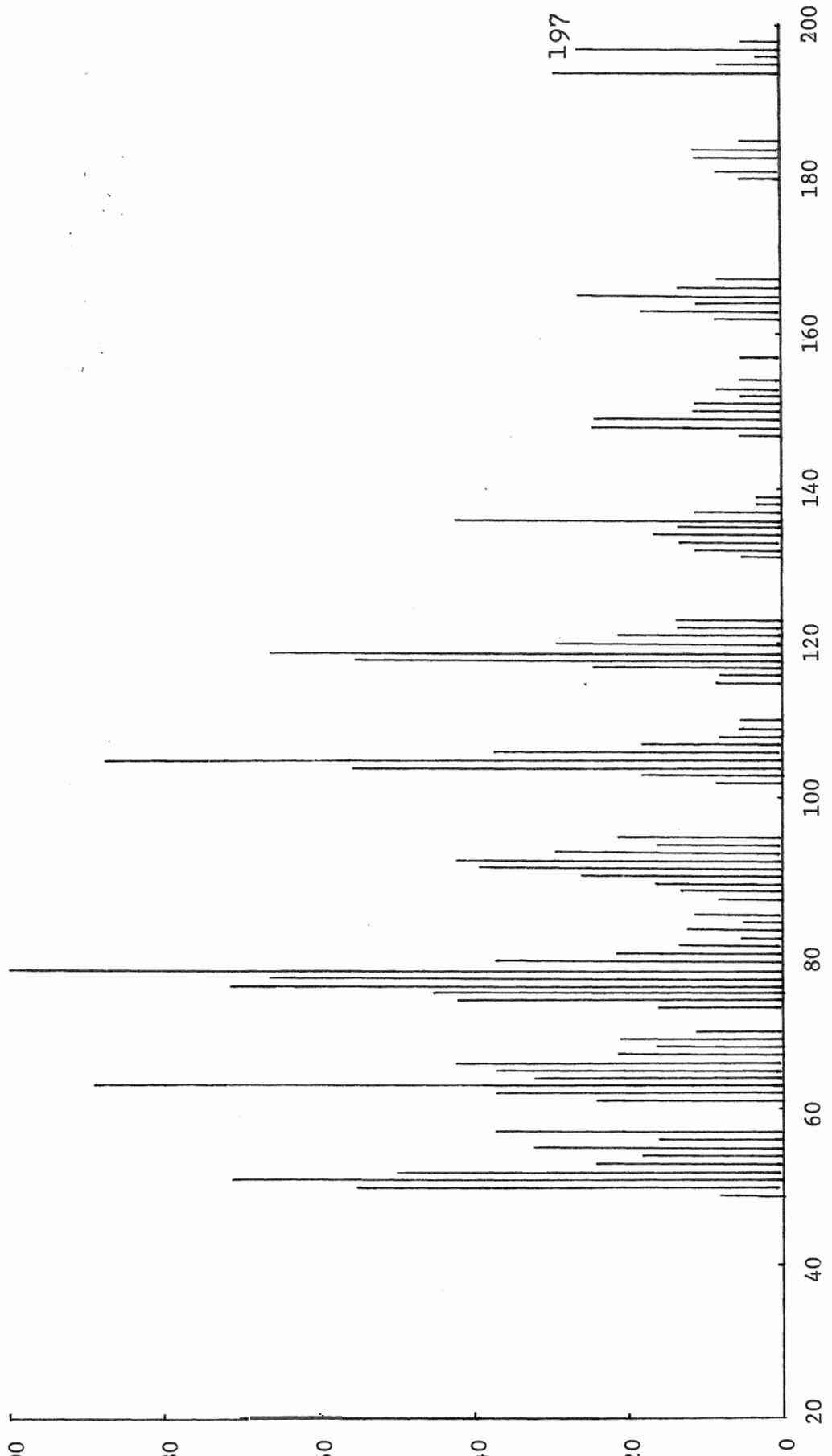

100
80
60
40
20
0
30
50
70
90
110
130
150
170
190
210
230
211

Fig.27. Mass spectrum of DNPMA


197
100
80
60
40
20
0
20
40
60
80
100
120
140
160
180
200